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(54) Title: POXVIRUS-BASED EXPRESSION VECTORS CONTAINING HETEROLOGOUS INSERTS DERIVED FROM LENTIVIRUSES

(57) Abstract

Recombinants containing and expressing lentivirus, retrovirus or immunodeficiency virus DNA and methods for making and using the same are disclosed and claimed. In an exemplified embodiment, attenuated recombinant viruses containing DNA encoding a feline immunodeficiency virus epitope such as an antigen, as well as methods and compositions employing the viruses, expression products therefrom, and antibodies generated from the viruses or expression products, are disclosed and claimed. The recombinants can be NYVAC or ALVAC recombinants. The DNA can encode at least one of: Env, Gag, Pol, or combinations thereof such as Gag and Pol or protease or Env, Gag and Pol or protease. The recombinants and gene products therefrom and antibodies generated by them have several preventive, therapeutic and diagnostic uses. DNA from the recombinants are useful as probes or, for generating PCR primers or for immunization. The immunogenicity and protective efficacy of immunization protocols involving ALVAC–FIV and priming with a recombinant canarypox virus ALVAC–FIV followed by a booster immunization with inactivated FIV–infected celled vaccine (ICV) was evaluated against FIV challenge in cats and the protocol was shown to effectively induce FIV–specific protective immune responses. Further, it was found that immunized cats were fully protected from an initial challenge with a slightly heterologous FIV strain ($50CID_{50}$) and were partially protected from a second challenge with a distinctly heterologous FIV strain ($75CID_{50}$) given eight months after the initial challenge without any intervening booster.

POXVIRUS–BASED EXPRESSION VECTORS CONTAINING HETEROLOGOUS INSERTS DERIVED FROM LENTIVIRUSES

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STATEMENT OF POSSIBLE GOVERNMENT RIGHTS

RELATED APPLICATIONS

15   Reference is made to U.S. application Serial
No. 08/417,210 filed April 5, 1995 as a continuation-in-
part of application Serial No. 08/223,842, filed April 6,
1994 which in turn is a continuation-in-part of
20   application Serial No. 07/897,382, filed June 11, 1992
(now U.S. application Serial No. 08/303,275, filed
September 7, 1994), which in turn is a continuation-in-
part of application Serial No. 07/715,921, filed June 14,
1991. Application Serial No. 08/417,210 is also a
25   continuation-in-part of application Serial No.
08/105,483, filed August 13, 1993, now U.S. Patent No.
5,494,807, which in turn is a continuation of application
Serial No. 07/847,951, filed March 6, 1992, which in turn
is a continuation-in-part of application Serial No.
30   07/713,967, filed June 11, 1991, which in turn is a
continuation in part of application Serial No.
07/666,056, filed March 7, 1991 (now U.S. Patent No.
5,364,773). Mention is also made of co-pending allowed
application Serial No. 08/184,009, filed January 19, 1994
35   as a continuation-in-part of application Serial No.
08/007,115, filed January 20, 1993. Each of the

aforementioned and above-referenced applications and
patent(s) are hereby incorporated herein by reference.

FIELD OF THE INVENTION

5   The present invention relates: to certain
product(s) from lentivirus, retrovirus and/or
immunodeficiency virus, e.g., HIV, SIV, EIAV, BIV, FIV,
comprising certain epitope(s) of interest, preferably
Env, Gag, Pol, and accessory gene products, e.g. Tat,
Rev, more preferably of Gag and Pol or Env, Gag and Pol
10   and most preferably Gag and protease; to certain nucleic
acid molecule(s), e.g., RNA, DNA, encoding the
product(s); to a vector, preferably a mammalian vector
system, comprising the nucleic acid molecule(s) and
preferably expressing the product(s) as exogenous to the
15   vector; to the product(s) obtained or obtainable from
expression by the vector; to immunological, immunogenic
and/or vaccine compositions comprising the vector and/or
the product(s); to methods for preparing the product(s);
to methods for preparing the vector; to methods for
20   preparing the compositions; and to methods for using the
product(s), vector and compositions, including methods
for obtaining an immunological response such as by
immunization regimens wherein the product(s), vector
and/or compositions are administered alone or in a
25   prime/boost configuration with inactivated lentivirus,
retrovirus or recombinant subunit preparations, e.g., in
a prime/boost configuration with an inactivated infected
cell vaccine or immunological or immunogenic composition
(ICV).

30   The invention especially relates to recombinant
immunological, immunogenic or vaccine compositions and
their utility in stimulating a response, such as
providing protection against a lentivirus challenge
exposure, including exposure to a heterologous strain.
35   The recombinant composition is preferably comprised of a
mammalian vector system expressing lentivirus gene
products used in effective immunization regimens alone or

in a prime/boost configuration with inactivated
lentivirus preparations (e.g., ICV) or recombinant
subunit preparations.

5   Several documents are referenced in this
application. Full citation to these documents is found
at the end of the specification immediately preceding the
claims or where the document is mentioned; and each of
these documents is hereby incorporated herein by
reference.

10   **BACKGROUND OF THE INVENTION**

The patent and scientific literature includes
various mammalian vector systems such as mammalian virus-
based vector systems and mammalian DNA-based vector
systems, and how to make and use these vector systems,
15   for instance for cloning of exogenous DNA and expression
of proteins, as well as uses for such proteins and uses
for products from such proteins.

For instance, recombinant poxvirus (e.g.,
vaccinia, avipox virus) and exogenous DNA for expression
20   in this viral vector system can be found in U.S. Patent
Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941,
5,338,683, 5,494,807, 5,503,834, 4,722,848, 5,514,375,
U.K. Patent GB 2 269 820 B, WO 92/22641, WO 93/03145, WO
94/16716, PCT/US94/06652, and allowed U.S. application
25   Serial No. 08/184,009, filed January 19, 1994. See
generally Paoletti, "Applications of pox virus vectors to
vaccination: An update," PNAS USA 93:11349-11353, October
1996; Moss, "Genetically engineered poxviruses for
recombinant gene expression, vaccination, and safety,"
30   PNAS USA 93:11341-11348, October 1996.

Baculovirus expression systems and exogenous
DNA for expression therein, and purification of
recombinant proteins therefrom can be found in
Richardson, C.D. (Editor), Methods in Molecular Biology
35   39, "Baculovirus Expression Protocols" (1995 Humana Press
Inc.) (see, e.g., Ch.18 for influenza HA expression,
Ch.19 for recombinant protein purification techniques),

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Smith et al., "Production of Huma Beta Interferon in
Insect Cells Infected with a Baculovirus Expression
Vector," Molecular and Cellular Biology, Dec., 1983, Vol.
3, No. 12, p. 2156-2165; Pennock et al., "Strong and
5   Regulated Expression of *Escherichia coli* B-Galactosidase
in Infect Cells with a Baculovirus vector," Molecular and
Cellular Biology Mar. 1984, Vol. 4, No. 3, p. 399-406;
EPA 0 370 573 (Skin test and test kit for AIDS,
discussing baculovirus expression systems containing
10   portion of HIV-1 env gene, and citing U.S. application
Serial No. 920,197, filed October 16, 1986 and EP Patent
publication No. 265785).
          U.S. Patent No. 4,769,331 relates to
herpesvirus as a vector. See also Roizman, "The function
15   of herpes simplex virus genes: A primer for genetic
engineering of novel vectors," PNAS USA 93:11307-11312,
October 1996; Andreansky et al., "The application of
genetically engineered herpes simplex viruses to the
treatment of experimental brain tumors," PNAS USA
20   93:11313-11318, October 1996. Epstein-Barr virus vectors
are also known. See Robertson et al. "Epstein-Barr virus
vectors for gene delivery to B lymphocytes," PNAS USA
93:11334-11340, October 1996. Further, there are
alphavirus-based vector systems. See generally Frolov et
25   al., "Alphavirus-based expression vectors: Strategies and
applications," PNAS USA 93:11371-11377, October 1996.
          There are also poliovirus and adenovirus vector
systems (see, e.g., Kitson et al., J. Virol. 65, 3068-
3075, 1991; Grunhaus et al., 1992, "Adenovirus as cloning
30   vectors," Seminars in Virology (Vol. 3) p. 237-52, 1993;
Ballay et al. EMBO Journal, vol. 4, p. 3861-65; Graham,
Tibtech 8, 85-87, April, 1990; Prevec et al., J. Gen
Virol. 70, 429-434). See also U.S. applications Serial
Nos. 08/675,556 and 08/675,566, filed July 3, 1996
35   (adenovirus vector system, preferably CAV2) and PCT
WO91/11525 (CAV2 modified to contain a promoter-gene
sequence within the region from the SmaI site close to

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the end of the inverted terminal repeat region up to the promoter for the early region 4 (E4)).

There are also DNA vector systems. As to transfecting cells with plasmid DNA for expression therefrom, reference is made to Felgner et al. (1994), J. Biol. Chem. 269, 2550-2561. As to direct injection of plasmid DNA as a simple and effective method of vaccination against a variety of infectious diseases reference is made to Science, 259:1745-49, 1993. See also McClements et al., "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease," PNAS USA 93:11414-11420, October 1996.

In 1983, human immunodeficiency virus type 1 (HIV1) was identified as the causative agent of AIDS and was subsequently classified into the lentivirus subfamily of the retrovirus family (Hardy, 1990). Other members of the lentivirus subfamily are equine infectious anemia virus (EIAV), feline immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV), Simian immunodeficiency virus (SIV) and HIV-2. Much attention within the field of medical virology has been focused on the AIDS pandemic caused by infection with HIV. This lentivirus system has been scrutinized with respect to its molecular biology, immunobiology and pathogenesis in an effort to develop safe and effective vaccines and antiviral therapies. To date, HIV, as well as other lentiviral vaccine studies using different vaccine types have encountered varying degrees of success (Heeney et al., 1994; Daniel et al., 1992; Fultz et al., 1992; Girard et al., 1991; Issel et al., 1992). Further, knowledge is still lacking on the relevance of specific HIV immune responses on vaccine efficacy in humans. Thus, after many years, despite a massive, worldwide effort, an effective HIV1 vaccine is still not available.

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Infection of cats with feline immunodeficiency virus (FIV) causes persistent infection and AIDS-like immunosuppressive diseases similar to the HIV infection. As such, FIV infection of cats provides a model for

5   investigating lentivirus immunopathogenicity and vaccine development (Pedersen et al., 1987; Johnson et al., 1994). Similar to HIV, heterogeneity exists, such that multiple FIV subtypes exist (Sadora et al., 1994; Okada et al., 1994). Indeed, like HIV, FIV strains have been

10   classified into four subtypes (A-D) based on genetic differences predominantly in the env and, to a lesser extent gag coding regions.

Thus, while inactivated whole FIV vaccines and inactivated FIV-infected cell vaccines (ICV) have

15   obtained protection against homologous and slightly heterologous FIV (Hosie et al., 1995;Johnson et al., 1994; Yamamoto et al., 1991, 1993), these same vaccines failed to induce protective immunity against distinctly heterologous FIV strains of other subtypes such that

20   induction of protective immunity against a broad range of FIV subtypes may call for a modified or different vaccine approach. This obviously raises concerns relevant to vaccine development. It must also be noted that the FIV prevalence in the cat population is greater than HIV is

25   in man (Verschoor et al., 1996). The development of an FIV vaccine or immunogenic composition is not only useful in providing a model for an HIV vaccine or immunogenic composition but is also therefore of importance from a veterinary health perspective.

30   More particularly, from the previous FIV studies (Hosie et al., 1995; Johnson et al., 1994; Yamamoto et al., 1991, 1993) it was observed that only cats with significant FIV Env-specific serum reactivity were likely to be protected against homologous challenge

35   exposure. In no case were vaccine-administered animals lacking such a response observed to be protected against FIV challenge (Johnson et al., 1994; Yamamoto et al.,

1991, 1993). Together, these results coupled to the
observations, to date, that subunit immunogens have not
been shown to elicit a protective immune response in
target species bring to the forefront several important
5   points relevant to the state-of-the-art for FIV and
lentivirus, vaccine development in general. One
exception perhaps is with the simian immunodeficiency
virus (SIV)/macaque system where certain recombinant
subunit preparations (including vaccinia-based
10   recombinants) or
combinations of these recombinant subunits have
conferred, at least, partial protection from SIV
challenge exposure (Hu, 1992; 1994; 1995). This data is
somewhat limited in scope since complete protection from
15   infection was not observed and challenge studies were not
performed with a distinctly heterologous SIV strain.
Moreover, no level of protection was afforded by
recombinant subunits devoid of an SIV Env component (Hu
et al., 1994).
20   Relevant to FIV vaccine development, no sub-
unit based vaccine candidate has been taught or
suggested; there is no teaching as to how to develop a
subunit vaccine; and, it is not obvious as to how to
develop a subunit-based vaccine candidate.
25   Secondly, a different or perhaps modified
approach, as compared to the inactivated conventional
vaccines, needs to be developed to afford protection
against heterologous strains (Hosie et al.,1995;Johnson
et al., 1994).
30   Lastly, Env-specific immune responses in
protective immunity may be important (Johnson et al.,
1994; Yamamoto et al., 1991, 1993). Indeed, in Flynn et
al., "ENV-specific CTL Predominate in Cats Protected from
Feline Immunodeficiency Virus Infection by Vaccination,"
35   *The Journal of Immunology*, 1996, 157:3658-3665, at 3664
the authors conclude "that FIV Env-specific CTL may be
more effective in protective immunity to FIV infection of

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domestic cats" such that "future vaccine strategies
should be aimed at eliciting both humoral and cell-
mediated immune responses that are long-lived, recognize
appropriate epitopes on the viral envelope glycoprotein,
and are targeted to tissues known to sequester virus."

It can thus be appreciated that provision of a
feline immunodeficiency virus recombinant subunit
immunogenic, immunological or vaccine composition which
induces an immunological response against feline
immunodeficiency virus infections when administered to a
host, e.g., a composition having enhanced safety such as
NYVAC- or ALVAC- based recombinants containing exogenous
DNA coding for an FIV epitope of interest, such as of FIV
Env, Gag, or Pol, especially in an immunogenic
configuration, or any combination thereof, for instance,
FIV Gag-protease, Gag-Pol, or Gag and a portion of Pol
(such as a portion of Pol including protease) or all of
Env, Gag and Pol or a portion of Pol, in combination,
would be a highly desirable advance over the current
state of technology. Further, use of such recombinants
or compositions containing such recombinants in a prime-
boost regimen, e.g., wherein the recombinant composition
is used in an initial immunization and a subsequent
immunization is with an inactivated FIV, or ICV, or other
recombinant subunit preparation would be a highly
desirable advance over the current state of technology.

And more generally, it can thus be appreciated
that provision of a lentivirus, retrovirus or
immunodeficiency virus recombinant subunit immunogenic,
immunological or vaccine composition which induces an
immunological response against the lentivirus, retrovirus
or immunodeficiency virus infections when administered to
a host, e.g., a composition having enhanced safety such
as NYVAC- or ALVAC- based recombinants containing
exogenous DNA coding for a lentivirus, retrovirus, or
immunodeficiency virus epitope of interest, such as Env,
Gag, or Pol, especially in an immunogenic configuration,

or any combination thereof, for instance, Gag-protease,
Gag-Pol or Gag and a portion of Pol (such as a portion
including protease) all of Env, Gag and Pol or a portion
of Pol, in combination such as Env, Gag-protease, in
5   combination, would be a highly desirable advance over the
current state of technology. Further, use of such
recombinants or compositions containing such recombinants
in a prime-boost regimen, e.g., wherein the recombinant
composition is used in an initial immunization and a
10   subsequent immunization is with an inactivated
lentivirus, retrovirus or immunodeficiency virus, or ICV,
or other recombinant subunit preparation, such as a
respective inactivated virus, ICV or other recombinant
subunit preparation would be a highly desirable advance
15   over the current state of technology (As to "respective",
if the recombinant is, for example an FIV recombinant,
inactivated FIV or an FIV ICV preparation may be
"respective").

OBJECTS AND SUMMARY OF THE INVENTION

20   It is therefore an object of the invention to
provide certain product(s) from lentivirus, retrovirus
and/or immunodeficiency virus, e.g., HIV, SIV, EIAV, BIV,
FIV, Visna virus, carpine arthritis-encephalitis virus,
comprising certain epitope(s) of interest, preferably
25   Env, Gag, Pol or epitopes thereon, with optional
accessory functions or proteins or epitope(s) of interest
thereon e.g. Tat and/or Rev, more preferably Gag and Pol,
or Env, Gag and Pol, or Gag and a portion of Pol, or Env,
Gag and a portion of Pol, especially such a portion
30   including protease, and most preferably Gag and protease,
or Env, Gag and protease, or epitopes thereon, with
optional accessory functions or proteins, e.g., Tat
and/or Rev or other such functions/proteins, or epitopes
thereon. Other accessory functions or proteins which can
35   be included in the product(s) or epitope(s) of interest
include any or all of net, vpu, vit, vpr, and vpx or
epitope(s) thereon, _inter alia_; see Trono, D., Cell,

82:189-192, July 28, 1995. Such accessory functions or
proteins may be considered non-envelope functions or
proteins which can be included in the product(s) or
epitope(s) of interest, e.g. for induction of a cellular
5   response. For instance, for a given lentivirus,
retrovirus or immunodeficiency virus pathogen, that
pathogen's accessory function(s) or protein(s) or
epitope(s) thereon can be included; for example, the
product(s) could thus include Gag-Pro plus accessory
10   function(s) or protein(s) or epitope(s) thereon.

It is an additional object of the invention to
provide certain nucleic acid molecule(s), e.g., RNA, DNA,
encoding the product(s), e.g., encoding certain
epitope(s) of interest such as Gag and protease or all of
15   Env, Gag and Pol.

It is a further object of the invention to
provide a vector, preferably a mammalian vector system,
comprising the nucleic acid molecule(s) and preferably
expressing the product(s) as exogenous to the vector,
20   e.g., a poxvirus, baculovirus, herpesvirus, Epstein-Barr,
alphavirus, poliovirus, adenovirus or DNA vector system.

It is a further object of the invention to
provide the product(s) obtained or obtainable from
expression by the vector.

25   It is yet a further object of the invention to
provide an immunological, immunogenic and/or vaccine
composition comprising the vector and/or the product(s).

It is still another object of the invention to
provide methods for preparing the product(s).

30   It is yet another object of the invention to
provide methods for preparing the vector.

It is even still a further object of the
invention to provide methods for preparing the
compositions.

35   And it is a further object of the invention to
provide methods for using the product(s), vector and
compositions, including methods for obtaining an

immunological response such as by immunization regimens
wherein the product(s), vector and/or compositions are
administered alone or in a prime/boost configuration with
inactivated lentivirus, retrovirus or recombinant subunit
5   preparations, e.g., in a prime/boost configuration with
an inactivated infected cell vaccine or immunological or
immunogenic composition (ICV), such as a respective ICV.

The present invention thus relates to
10 recombinant immunological, immunogenic or vaccine
compositions and their utility in eliciting a response
such as by providing protection against lentivirus,
retrovirus or immunodeficiency virus challenge exposure
in a target species.
15   More in particular, the invention relates to a
mammalian vector system for the insertion and expression
of foreign genes for use as safe immunization vehicles to
elicit a response such as a protective immune response
against lentiviruses, retroviruses or immunodeficiency
20 viruses.
In accord with the herein objects, the
invention accordingly relates to a mammalian vector
system, which expresses gene products (e.g., a gene
product including an epitope of interest) of a
25 lentivirus, retrovirus or immunodeficiency virus such
EIAV, FIV, BIV, HIV, or SIV, with feline immunodeficiency
virus (FIV) presently preferred; and, the invention
relates to immunogenic and/or immunological and/or
vaccine compositions which induce an immunological and/or
30 protective response against a lentivirus, retrovirus or
immunodeficiency virus such EIAV, FIV, BIV, HIV, or SIV
exposure when administered to the target host, e.g., FIV
and a feline, such as a domesticated cat or kitten.
In one aspect, in furthermore of the herein
35 objects, the present invention comprises a mammalian
vector (e.g., poxvirus, baculovirus, herpesvirus,
Epstein-Barr, alphavirus, poliovirus, adenovirus or DNA

vector system, preferably a poxvirus) expressing a
lentivirus, retrovirus or immunodeficiency virus epitope
of interest, e.g., EIAV, FIV, BIV, HIV, or SIV,
preferably FIV; and, the epitope of interest is
5   preferably Gag/protease. The vector is useful in the
protection of the target species (e.g., feline) against a
highly homologous challenge exposure; and accordingly,
the invention encompasses an immunological, immunogenic
or vaccine composition comprising the vector and
10 optionally an acceptable carrier or diluent.

        In another aspect, in accordance with the
herein objects, the present invention comprises a method
for inducing an immunological response, preferably a
protective response comprising administering the vector
15 or composition comprising the vector to a host. The
method can be an immunizing regimen, e.g., priming with
the vector or composition comprising the vector (and
expressing the lentivirus, retrovirus or immunodeficiency
virus (e.g., FIV) epitope(s) of interest gene products)
20 and boosting with a respective lentivirus, retrovirus or
immunodeficiency subunit preparation (e.g., FIV
inactivated whole cell (ICV) preparation) or with a
respective lentivirus, retrovirus or immunodeficiency
recombinant subunit preparation (e.g., FIV epitope(s) of
25 interest from isolating such from expression of a
recombinant containing exogenous nucleic acid molecule(s)
encoding the same) to elicit an immunological response
such as conferring protection to the host (e.g., cats)
against homologous and heterologous lentivirus,
30 retrovirus or immunodeficiency virus isolates.

        It is an additional object of this invention to
provide a recombinant poxvirus antigenic, vaccine or
immunological composition having an increased level of
safety compared to known recombinant poxvirus vaccines.
35   It is a further object of this invention to
provide a modified vector for expressing a gene product

in a host, wherein the vector is modified so that it has
attenuated virulence in the host.

It is another object of this invention to
provide a method for expressing a gene product in a cell

5   cultured *in vitro* using a modified recombinant virus or
modified vector having an increased level of safety.

These and other objects and advantages of the
present invention will become more readily apparent after
consideration of the following.

10   In a further aspect, the present invention
relates to a vector, preferably a modified recombinant
virus having inactivated virus-encoded genetic functions
so that the recombinant virus has attenuated virulence
and enhanced safety. The functions can be non-essential,

15   or associated with virulence (e.g., essential). The
virus is advantageously a poxvirus, particularly a
vaccinia virus or an avipox virus, such as fowlpox virus
and canarypox virus. The vector which is preferably a
modified recombinant virus can include, within an

20   essential or nonessential region of the virus genome, a
heterologous DNA sequence which encodes an antigen or
epitope derived from a lentivirus, retrovirus or
immunodeficiency virus, e.g., EIAV, FIV, BIV, HIV, or
SIV, preferably feline immunodeficiency virus, such as,

25   e.g., Env, Gag, Pol, accessory functions (e.g. Tat, Rev),
or any combination thereof, such as Gag-Pol or Env, Gag,
and Pol or Gag and a portion of Pol or Env, Gag and a
portion of Pol, such as a portion of Pol including
protease, or Gag-protease or Env, Gag, and protease.

30   In another aspect, the present invention
relates to an antigenic, immunological, immunogenic or
vaccine composition or a therapeutic composition for
inducing an antigenic or immunological or protective
response in a host animal such as a feline, e.g.,

35   domesticated cat or kitten, inoculated with the
composition, the composition can include a carrier and an
inventive vector which is preferably a modified

recombinant virus having inactivated nonessential virus-
encoded genetic functions so that the recombinant virus
has attenuated virulence and enhanced safety; or the
expression product of such a vector or modified
5   recombinant virus. The virus used in the composition (or
for expressing a product for use in a composition)
according to the present invention is advantageously a
poxvirus, particularly a vaccinia virus or an avipox
virus, such as fowlpox virus and canarypox virus. The
10 modified recombinant virus can include, within an
essential or nonessential region of the virus genome, a
heterologous DNA sequence which encodes an antigenic
protein, e.g., an epitope of interest derived from a
lentivirus, retrovirus or immunodeficiency virus, e.g.,
15 EIAV, FIV, BIV, HIV, or SIV, preferably feline
immunodeficiency virus, such as an antigen, e.g., Env,
Gag, protease, or any combination thereof, such as Gag-
protease or Env, Gag, and protease.

In yet another aspect, the present invention
20 relates to a method for inducing an antigenic,
immunological, immunogenic, vaccine (protective), and/or
therapeutic response in a host animal such as a feline,
e.g., domesticated cat or kitten, for instance, a host
animal in need of such a response, comprising
25 administering an amount of the aforementioned inventive
composition effective to obtain the response, either
alone or as part of a prime-boost regimen (e.g.,
administering the inventive composition or administering
either or both of an inactivated lentivirus, retrovirus
30 or immunodeficiency virus or ICV or IWV either before or
after administering the inventive composition).

In a further aspect, the present invention
relates to a method for expressing a gene product in a
cell *in vitro* by introducing into the cell an inventive
35 vector, such as a modified recombinant virus having
attenuated virulence and enhanced safety. The vector or
modified recombinant virus can include, within a

nonessential or essential region of the virus genome, a
heterologous DNA sequence which encodes an epitope of
interest such as an antigenic protein, e.g. derived from
a retrovirus, lentivirus or immunodeficiency virus, e.g.,
5   EIAV, FIV, BIV, HIV, or SIV, preferably feline
immunodeficiency virus, such as, e.g., Env, Gag, Pol,
accessory functions (e.g. Tat, Rev), or any combination
thereof, such as Gag-Pol, or Env, Gag and Pol, or Gag and
a portion of Pol or Env, Gag or a portion of Pol wherein
10 the portion can include protease, or Gag-protease or Env,
Gag, protease. The gene product can be harvested from
the cells, or the cells can then be reinfused directly
into an animal or used to amplify specific reactivities
for reinfusion (*Ex vivo* therapy).
15   Thus, in a specific further aspect, the present
invention relates to a method for expressing a gene
product in a cell cultured *in vitro* by introducing into
the cell a vector or preferably a modified recombinant
virus having attenuated virulence and enhanced safety.
20 The vector or modified recombinant virus can include,
within an essential or nonessential region of the virus
genome, a heterologous DNA sequence which encodes an
epitope of interest or an antigenic protein, e.g.,
derived from a lentivirus, retrovirus, or
25 immunodeficiency virus, e.g., EIAV, FIV, BIV, HIV, or
SIV, preferably feline immunodeficiency virus, such,
e.g., Env, Gag, Pol, accessory functions (e.g. Tat,
Rev), or any combination thereof, such as Gag-Pol, or
Env, Gag and Pol, or Gag and a portion of Pol, or Env,
30 Gag and a portion of Pol, wherein the portion can include
a protease, or Gag-protease or Env, Gag, protease. The
product can then be administered to a host to stimulate a
response.
            Antibodies can be raised by compositions
35 including the inventive vectors or recombinants or
expression products of the inventive vectors or
recombinants. The antibodies raised can be useful in a

host for the prevention or treatment of a lentivirus, retrovirus or immunodeficiency virus such as feline immunodeficiency virus. The antibodies or the expression products of the inventive vectors or recombinants can be

5   used in diagnostic kits, assays or tests to determine the presence or absence in a sample such as sera of lentivirus, retrovirus or immunodeficiency virus, e.g., feline immunodeficiency virus, or antigens thereof or antibodies thereto or of recombinants of the present

10 invention. Accordingly, an aspect of the invention involve the antibodies, diagnostic kits, assays, or tests.

          In a still further aspect, the present invention relates to a modified recombinant virus having

15 nonessential or essential virus-encoded genetic functions inactivated therein so that the virus has attenuated virulence, and wherein the modified recombinant virus further contains DNA from a heterologous source in an essential or nonessential region of the virus genome.

20 The DNA can code for an antigen or epitope of interest of a lentivirus, retrovirus or immunodeficiency virus, e.g., EIAV, FIV, BIV, HIV, or SIV, preferably feline immunodeficiency virus, e.g., Env, Gag, Pol, accessory functions, or any combination thereof, such as Gag-Pol or

25 Env, Gag and Pol, or Gag and a portion of Pol or Env, Gag and a portion of Pol, wherein the portion of Pol can include protease, or Gag-protease or Env, Gag, protease. In particular, the genetic functions are inactived by deleting or disrupting an open reading frame encoding a

30 virulence factor, e.g., an essential region, or by utilizing naturally host restricted viruses, especially naturally host restricted viruses displaying attenuated virulence from having been serial passages and/or plaque purification (with or without subsequent passages). The

35 virus used according to the present invention is advantageously a poxvirus, particularly a vaccinia virus

or an avipox virus, such as fowlpox virus and canarypox
virus.

Advantageously, the open reading frame is
selected from the group consisting of J2R, B13R + B14R,
5   A26L, A56R, C7L - K1L, and I4L (by the terminology
reported in Goebel et al., 1990a,b); any combination
thereof and, preferably the combination thereof. In this
respect, the open reading frame comprises a thymidine
kinase gene, a hemorrhagic region, an A type inclusion
10 body region, a hemagglutinin gene, a host range gene
region or a large subunit, ribonucleotide reductase; or,
any combination thereof and preferably the combination
thereof. The modified Copenhagen strain of vaccinia
virus is identified as NYVAC (Tartaglia et al., 1992).
15 NYVAC and NYVAC variations have essential regions deleted
or disrupted therein, and for a subsequent publication to
Tartaglia et al., 1992 which like Tartaglia et al., 1992
relates to deletion of essential regions of vaccinia
virus (and therefore relates to NYVAC, NYVAC variations,
20 or viruses taught by or obvious from viruses of Tartaglia
et al., 1992 such as NYVAC and NYVAC variations) e.g.,
NYVAC.1, NYVAC.2, reference is made to PCT WO 95/30018.
With respect to NYVAC and NYVAC variations, reference is
also made to U.S. Patents Nos. 5,364,773 and 5,494,807.
25 However, other vaccinia virus strains, such as the COPAK
strain, can also be used in the practice of the
invention.

In another preferred embodiment the vector is
an attenuated canarypox virus, such as a canarypox virus
30 which is not a mixed population. For instance, a
Rentschler vaccine strain which was attenuated through
more than 200 serial passages on chick embryo
fibroblasts, a master seed therefrom was subjected to
four successive plaque purifications under agar, from
35 which a plaque clone was amplified through five
additional passages. Such a canarypox is called ALVAC.

Results of restriction digests of plaque
derived from Kanapox is as follows: Genomic DNA isolated
from plaque isolates number 1, 4, and 5 cloned from
canarypox virus (Kanapox). DNA from these plaques and
5   from the uncloned canarypox virus was digested with
restriction enzymes HindIII, BamHI and EcoRI and run on a
0.8% agarose gel. The gel was stained with ethidium
bromide and photographed under UV light (see Fig. 1).
The lanes are labeled v = uncloned canarypox virus, 1 =
10   plaque 1, 4 = plaque 4, and 5 = plaque 4. A 1 kb
molecule weight marker was run on the far left lane. The
restriction profiles show distinct differences between
the various plaque isolates. Plaque 1 was chosen as
ALVAC (CP_{pp}).

15   In the restriction patterns of the uncloned
canarypox virus (lanes = v) several submolar bands can be
observed. However, when plaqued cloned, these submolar
bands (lanes labeled 1, 4 and 5) become molar species in
at least some of the plaque-cloned isolates. This
20   indicates that the uncloned canarypox virus (Kanapox)
represents a mixture of genomic variants which differ in
restriction profiles.

Thus, ALVAC is different from, has unique
properties over, and is superior to Kanapox; and
25   therefore, ALVAC is a preferred vector in the practice of
the invention. In particular, the Rentschler strain
canarypox virus (Kanapox), from restriction analyses,
represents a mixture of viral variants; that is, Kanapox,
from which ALVAC was derived, was a mixed population. It
30   is not unprecedented for a vaccine preparation, such as
Kanapox, to contain multiple variants. ALVAC is not a
mixed population. As such, ALVAC has several unique
properties which are not shared by Kanapox, for instance:

ALVAC has a uniform genetic background. This
35   property provides ALVAC with consistency; a unique
feature of being useful for preparing vector-based
vaccines. Consistency is useful for quality control and

regulatory considerations. This property of consistency
of ALVAC provides ALVAC with the ability to pass quality
control and regulatory considerations, i.e., be useful in
the development of vector-based vaccines with predicted
5   genetic properties.

Biological consistency is controlled using
ALVAC to derive recombinants. Kanapox does not provide
biological consistency. Indeed, Kanapox cannot
consistently provide an effective recombinant product.
10   Biological consistency and a consistently effective
recombinant product are useful; for instance, for a
consistent biological profile with respect to virulence,
with regard to virus/host interactions, and ultimately
for use as an immunization vehicle. ALVAC achieves
15   biological consistency and consistently effective
recombinant products. When Kanapox is used in deriving
recombinants, there is no control over the virus
background into which the foreign gene is inserted; and
therefore, the properties of the resultant recombinant
20   remain in question (cf. studies with vaccinia virus which
illustrate that not all vaccinia genetic backgrounds are
equivalent as immunization vehicles). ALVAC provides
certainty with respect to its virus background, its
properties related to virulence, and its functioning as
25   an immunization vehicle.

Although the present invention has primarily
been described using a canarypox virus (ALVAC)-based
vector, it should be understood that invention herein
also resides in the expression of specific lentivirus,
30   retrovirus or immunodeficiency virus gene products and
their utility for conferring an immune response such as a
protective immune response. Hence, the invention also
relates to alternative mammalian vector systems.
Examples of such vector systems include other poxviruses,
35   adenoviruses, herpesviruses, alphavirus-based systems,
bacterial expression systems, and DNA-based immunogen
formulations.

Although the present invention has primarily been described using the lentivirus FIV, it should be understood that invention herein also resides in the expression of functional homologs of the FIV gene
5   products from other lentivirus and retrovirus and immunodeficiency virus systems, e.g., Env, Gag/protease (i.e., Env, Gag and Pol or a portion of Pol, or Gag and Pol or a portion of Pol wherein the portion of Pol can include protease (without Env), or Env, Gag, protease or
10   Gag-protease (without Env) or Env, Gag, Pol or a portion of Pol and accessory functions (e.g. Tat, Rev) or Gag, Pol or a portion of Pol wherein the portion of Pol can include protease and accessory functions (without Env) or Env, Gag protease and accessory functions or Gag-protease
15   and accessory functions (without Env), of EIAV, FIV, BIV, HIV, or SIV, *inter alia*). Hence, the invention relates to other lentivirus systems including human immunodeficiency virus -1, -2 (HIV-1,-2), bovine immunodeficiency virus (BIV), equine infectious anemia
20   virus (EIAV), as well as other mammalian lentiviruses.

These and other embodiments are disclosed or are obvious from and encompassed by the following detailed description.

DEPOSITS

25   The following have deposited with the ATCC under the terms of the Budapest Treaty.

Material	Accession Number	Deposit Date
ALVAC	_____	NOV. 14, 1996
Plasmid MM 138 (pMM138)	_____	NOV. 14, 1996
(containing FIV env,		
gag/pro)		
Plasmid MM 129 (pMM129)	_____	NOV. 14, 1996
(containing FIV		
gag/pro)		

30

35   The invention thus comprehends nucleic acid molecules, including encoding product(s) having sequences as in the Deposited Material, as well as nucleic acid molecules having substantial homology thereto (e.g., at least 85% homology).

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings, incorporated herein by reference, in which:

FIG. 1 shows the results of plaque purifying Kanapox, as described above;

FIG. 2 shows the nucleotide sequence of FIV env from Rhone Merieux (SEQ ID NO:1) (The FIV env start codon is at position 1 and the stop codon is at position 2569. Plasmid ptg6184, containing the FIV env coding sequence, was from Rhone Merieux(Lyon, France). The FIV env coding sequence in ptg6184 was sequenced and the following differences with the sequence below were observed: position 1218 T is G in ptg6184 changing phe to leu; position 1220 G to A changes gly to glu; and position 2201 C to A change ala to glu);

FIG. 3 shows the nucleotide sequence of FIV gag/pol coding sequences from Rhone Merieux (SEQ ID NO:2) (The gag start codon is at position 1 and the gag stop codon is at position 1414. The ribosomal frameshift site is near position 1255. The frameshift is -1 in relation to the gag open reading frame. The frameshift goes into the pol open reading frame. The pol stop codon is at position 4614. Plasmid ptg8133 from Rhone Merieux contains the FIV gag/pol coding sequences. Part of ptg8133 has been sequenced and the CG at positions 577-578 below is GC in ptg8133, changing the codon from arg to ala);

FIG. 4 shows the ALVAC-nucleotide sequence comprised in the C6 donor plasmid pC6L (SEQ ID NO:3) (Plasmid pC6L contains the C6 insertion sites SmaI (position 409) and EcoRI (position 425));

FIG. 5 shows the predicted nucleotide sequence of the vCP242 insertion (SEQ ID NO:4) (The H6 promotor starts at position 55. The FIV env start codon is at

position 179, and the FIV _env_ stop codon is at position
2749). Positions 1 through 54 and positions 2750 through
2879 flank the H6/FIV _env_ expression cassette);

5   FIG. 6 shows the predicted nucleotide sequence
of I3L promoted FIV _gag_/protease expression cassette and
flanking regions in vCP253 (SEQ ID NO:5) (The I3L
promoter begins at position 135. The _gag_ start codon is
at position 235 and the protease stop codon is at
position 1648);

10   FIG. 7 shows the predicted nucleotide sequence
of the H6 promoted FIV _env_/I3L promoted FIV _gag_/protease
expression cassette and flanking regions in vCP255 (SEQ
ID NO:6) (The H6 promotor starts at position 129, the FIV
env start codon is at position 253, and the FIV _env_ stop

15 codon is at position 2823. The I3L promotor starts at
position 2830, the FIV _gag_ start codon is at position
2930 and the FIV _gag_ stop codon is at position 4282. The
ribosomal frameshift site is near position 4184. The
frameshift is -1 in relation to the _gag_ open reading

20 frame. The frameshift goes into the _pol_ open reading
frame. The stop codon for the protease gene is at
position 4641. Positions 1 through 128 and positions
4642 through 4727 flank the H6 FIV _env_/I3L FIV
gag/protease expression cassette); and

25   FIG. 8 shows the predicted nucleotide sequence
of vCP329 insertion (SEQ ID NO:7) (The H6 promoter starts
at position 2146. The coding sequence for FIV 97TM is
from position 2022 to position 42. The I3L promoter
starts at position 2253. The FIV _gag_ start codon is at

30 position 2353 and the _pol_ stop codon is at position
3766).

DETAILED DESCRIPTION OF THE INVENTION

        As discussed above, the invention specifically
relates to: vector-based lentivirus, retrovirus, or

35 immunodeficiency virus, e.g., EIAV, FIV, BIV, HIV, or
SIV, preferably feline immunodeficiency virus (FIV)
recombinants, preferably recombinants containing DNA

23

encoding an epitope(s) of interest, more preferably Env,
Gag, or Pol, or combinations thereof such as Gag and Pol
or a portion of Pol, or Env, Gag and Pol or a portion of
Pol or Gag and protease, or Env, Gag, and protease, with

5   an attenuated poxvirus such as TROVAC, NYVAC and ALVAC as
preferred poxvirus vectors (NYVAC and ALVAC being most
preferred, and ALVAC being especially preferred); and,
compositions containing the inventive recombinants or
expression products therefrom; and to methods for making

10   and using the inventive recombinants, expression products
therefrom and compositions including the recombinants
and/or expression products.

Thus, in a general way, the invention provides
a vector comprising exogenous DNA encoding at least one

15   lentivirus epitope. The epitope can be from a lentivirus
other than SIV. More preferably, the epitope is of Gag
and Pol or Env, Gag and Pol or Env, Gag and a portion of
Pol or Gag and a portion of Pol or Gag-protease, or Env,
Gag, and protease; and, most preferably the epitope is

20   Gag and protease or epitope(s) on Gag and protease which
elicit a response which is the same as or similar to Gag
and protease. And, the vector preferably induces an
immune response, more preferably a protective immune
response, when administered to a target species (a target

25   species is a host susceptible to the lentivirus; for
instance, felines such as domesticated cats and kittens
are a target species with respect to FIV).

The methods for making a vector or recombinant
can be by or analogous to the methods disclosed in U.S.

30   Patent Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941,
5,338,683, 5,494,807, 5,503,834, 4,722,848, 5,514,375,
U.K. Patent GB 2 269 820 B, WO 92/22641, WO 93/03145, WO
94/16716, PCT/US94/06652, allowed U.S. application Serial
No. 08/184,009, filed January 19, 1994, Paoletti,

35   "Applications of pox virus vectors to vaccination: An
update," PNAS USA 93:11349-11353, October 1996, Moss,
"Genetically engineered poxviruses for recombinant gene

expression, vaccination, and safety," PNAS USA 93:11341-
11348, October 1996, Richardson, C.D. (Editor), <u>Methods
in Molecular Biology 39</u>, "Baculovirus Expression
Protocols" (1995 Humana Press Inc.), Smith et al.,
5   "Production of Huma Beta Interferon in Insect Cells
Infected with a Baculovirus Expression Vector," Molecular
and Cellular Biology, Dec., 1983, Vol. 3, No. 12, p.
2156-2165; Pennock et al., "Strong and Regulated
Expression of *Escherichia coli* B-Galactosidase in Infect
10   Cells with a Baculovirus vector," Molecular and Cellular
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16, 1986, EP Patent publication No. 265785, U.S. Patent
No. 4,769,331, Roizman, "The function of herpes simplex
15   virus genes: A primer for genetic engineering of novel
vectors," PNAS USA 93:11307-11312, October 1996,
Andreansky et al., "The application of genetically
engineered herpes simplex viruses to the treatment of
experimental brain tumors," PNAS USA 93:11313-11318,
20   October 1996, Robertson et al. "Epstein-Barr virus
vectors for gene delivery to B lymphocytes," PNAS USA
93:11334-11340, October 1996, Frolov et al., "Alphavirus-
based expression vectors: Strategies and applications,"
PNAS USA 93:11371-11377, October 1996, Kitson et al., J.
25   Virol. 65, 3068-3075, 1991; Grunhaus et al., 1992,
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vol. 4, p. 3861-65, Graham, Tibtech 8, 85-87, April,
1990, Prevec et al., J. Gen Virol. 70, 429-434, U.S.
30   applications Serial Nos. 08/675,556 and 08/675,566, filed
July 3, 1996, PCT WO91/11525, Felgner et al. (1994), J.
Biol. Chem. 269, 2550-2561, Science, 259:1745-49, 1993
and McClements et al., "Immunization with DNA vaccines
encoding glycoprotein D or glycoprotein B, alone or in
35   combination, induces protective immunity in animal models
of herpes simplex virus-2 disease," PNAS USA 93:11414-
11420, October 1996.

Vaccinia virus and more recently other
poxviruses have been used for the insertion and
expression of foreign genes. A basic technique of
inserting foreign genes into live infectious poxvirus

5 involves recombination between pox DNA sequences flanking
a foreign genetic element in a donor plasmid and
homologous sequences present in the rescuing poxvirus
(Piccini et al., 1987).

Specifically, recombinant poxviruses can be

10 constructed in two steps known in the art and analogous
to the methods for creating synthetic recombinants of
poxviruses such as the vaccinia virus and avipox virus
described in U.S. Patent Nos. 4,769,330, 4,772,848,
4,603,112, 5,110,587, 5,179,993, 5,505,941, and

15 5,494,807, the disclosures of which, like the disclosures
of all documents cited herein, are incorporated herein by
reference.

First, the DNA gene sequence to be inserted
into the virus, e.g., an open reading frame from a non-

20 pox source, is placed into a plasmid construct such as an
E. coli plasmid construct into which DNA homologous to a
section of DNA of the poxvirus has been inserted.
Separately, the DNA gene sequence to be inserted can be
ligated to a promoter. The promoter-gene linkage is

25 positioned in the plasmid construct so that the promoter-
gene linkage is flanked on both ends by DNA homologous to
a DNA sequence flanking a region of pox DNA; for
instance, pox DNA containing a nonessential locus
(although an essential locus may also be used). The

30 resulting plasmid construct is then amplified, e.g., by
growth within *E. coli* bacteria (Clewell, 1972) and
isolated (Clewell et al., 1969; Maniatis et al., 1982).
Alternatively, the DNA gene sequence can, without
separate ligation to a promoter, merely be placed within

35 the plasmid construct so that the DNA gene sequence is
flanked on both ends by DNA homologous to a DNA sequence
flanking a region of pox DNA; for instance, a region

downstream from an endogenous promoter such that
expression of the gene sequence is under control of the
promoter and the promoter and coding portion of the DNA
gene sequence are thus adjacent.

5   Second, the isolated plasmid containing the DNA
gene sequence to be inserted is transfected into a cell
culture, e.g. chick embryo fibroblasts, along with the
poxvirus. Recombination between homologous pox DNA in
the plasmid and the viral genome respectively gives a

10 poxvirus modified by the presence, e.g., in a
nonessential region of its genome, of foreign DNA
sequences. The term "foreign" DNA designates exogenous
DNA, particularly DNA from a non-pox source, that codes
for gene products not ordinarily produced by the genome

15 into which the exogenous DNA is placed.

However, the foregoing is not meant to limit
means for obtaining vectors or recombinants of the
present invention, as any means for obtaining a vector or
recombinant e.g. a poxvirus-lentivirus, retrovirus,

20 and/or immunodeficiency virus, e.g., feline
immunodeficiency virus, recombinant may be used to obtain
the present invention.

Thus, genetic recombination is in general the
exchange of homologous sections of DNA between two

25 strands of DNA. In certain viruses RNA may replace DNA.
Homologous sections of nucleic acid are sections of
nucleic acid (DNA or RNA) which have the same sequence of
nucleotide bases.

Genetic recombination may take place naturally

30 during the replication or manufacture of new viral
genomes within the infected host cell. Thus, genetic
recombination between viral genes may occur during the
viral replication cycle that takes place in a host cell
which is co-infected with two or more different viruses

35 or other genetic constructs. A section of DNA from a
first genome is used interchangeably in constructing the
section of the genome of a second co-infecting virus in

which the DNA is homologous with that of the first viral
genome.

However, recombination can also take place
between sections of DNA in different genomes that are not
5   perfectly homologous. If one such section is from a
first genome homologous with a section of another genome
except for the presence within the first section of, for
example, a genetic marker or a gene coding for an
antigenic determinant inserted into a portion of the
10 homologous DNA, recombination can still take place and
the products of that recombination are then detectable by
the presence of that genetic marker or gene in the
recombinant viral genome. Accordingly, additional
strategies have recently been reported for generating
15 recombinant poxviruses such as recombinant vaccinia ·
virus; and, these strategies may be employed in the
practice of this invention.

Successful expression of the inserted DNA
genetic sequence by the modified infectious virus can
20 occur under two conditions. First, the insertion may be
into a nonessential region of the virus in order that the
modified virus remain viable, or into an essential region
whereby the essential function is not disturbed or the
function is not necessary for viability under all
25 conditions. A second condition for expression of
inserted DNA is the presence of a promoter in the proper
relationship to the inserted DNA. The promoter can be
located upstream from the coding portion of the DNA
sequence to be expressed.

30   Vaccinia virus has been used successfully to
immunize against smallpox, culminating in the worldwide
eradication of smallpox in 1980. In the course of its
history, many strains of vaccinia have arisen. These
different strains demonstrate varying immunogenicity and
35 are implicated to varying degrees with potential
complications, the most serious of which are post-

vaccinial encephalitis and generalized vaccinia
(Behbehani, 1983).

With the eradication of smallpox, a new role
for vaccinia became important, that of a genetically
5   engineered vector for the expression of foreign genes.
Genes encoding a vast number of heterologous antigens
have been expressed in vaccinia, often resulting in
protective immunity against challenge by the
corresponding pathogen (reviewed in Tartaglia et al.,
10   1990a).

The genetic background of the vaccinia vector
has been shown to affect the protective efficacy of the
expressed foreign immunogen. For example, expression of
Epstein Barr Virus (EBV) gp340 in the Wyeth vaccine
15   strain of vaccinia virus did not protect cottontop
tamarins against EBV virus induced lymphoma, while
expression of the same gene in the WR laboratory strain
of vaccinia virus was protective (Morgan et al., 1988).

A fine balance between the efficacy and the
20   safety of a vaccinia virus-based recombinant vaccine
candidate is extremely important. The recombinant virus
must present the immunogen(s) in a manner that elicits a
protective immune response in the vaccinated animal but
lacks any significant pathogenic properties. Therefore
25   attenuation of the vector strain would be a highly
desirable advance over the current state of technology.

A number of vaccinia genes have been identified
which are non-essential for growth of the virus in tissue
culture and whose deletion or inactivation reduces
30   virulence in a variety of animal systems.

The gene encoding the vaccinia virus thymidine
kinase (TK) has been mapped (Hruby et al., 1982) and
sequenced (Hruby et al., 1983; Weir et al., 1983).
Inactivation or complete deletion of the thymidine kinase
35   gene does not prevent growth of vaccinia virus in a wide
variety of cells in tissue culture. TK$^-$ vaccinia virus

is also capable of replication *in vivo* at the site of
inoculation in a variety of hosts by a variety of routes.

It has been shown for herpes simplex virus type
2 that intravaginal inoculation of guinea pigs with TK⁻

5   virus resulted in significantly lower virus titers in the
spinal cord than did inoculation with TK⁺ virus
(Stanberry et al., 1985). It has been demonstrated that
herpesvirus encoded TK activity *in vitro* was not
important for virus growth in actively metabolizing

10 cells, but was required for virus growth in quiescent
cells (Jamieson et al., 1974).

Attenuation of TK⁻ vaccinia has been shown in
mice inoculated by the intracerebral and intraperitoneal
routes (Buller et al., 1985). Attenuation was observed

15 both for the WR neurovirulent laboratory strain and for
the Wyeth vaccine strain. In mice inoculated by the
intradermal route, TK⁻ recombinant vaccinia generated
equivalent anti-vaccinia neutralizing antibodies as
compared with the parental TK⁺ vaccinia virus, indicating

20 that in this test system the loss of TK function does not
significantly decrease immunogenicity of the vaccinia
virus vector. Following intranasal inoculation of mice
with TK⁻ and TK⁺ recombinant vaccinia virus (WR strain),
significantly less dissemination of virus to other

25 locations, including the brain, has been found (Taylor et
al., 1991a).

Another enzyme involved with nucleotide
metabolism is ribonucleotide reductase. Loss of virally
encoded ribonucleotide reductase activity in herpes

30 simplex virus (HSV) by deletion of the gene encoding the
large subunit was shown to have no effect on viral growth
and DNA synthesis in dividing cells *in vitro*, but
severely compromised the ability of the virus to grow on
serum starved cells (Goldstein et al., 1988). Using a

35 mouse model for acute HSV infection of the eye and
reactivatable latent infection in the trigeminal ganglia,
reduced virulence was demonstrated for HSV deleted of the

large subunit of ribonucleotide reductase, compared to
the virulence exhibited by wild type HSV (Jacobson et
al., 1989).

5   Both the small (Slabaugh et al., 1988) and
large (Schmidtt et al., 1988) subunits of ribonucleotide
reductase have been identified in vaccinia virus.
Insertional inactivation of the large subunit of
ribonucleotide reductase in the WR strain of vaccinia
virus leads to attenuation of the virus as measured by
10   intracranial inoculation of mice (Child et al., 1990).

The vaccinia virus hemagglutinin gene (HA) has
been mapped and sequenced (Shida, 1986). The HA gene of
vaccinia virus is nonessential for growth in tissue
culture (Ichihashi et al., 1971). Inactivation of the HA
15   gene of vaccinia virus results in reduced neurovirulence
in rabbits inoculated by the intracranial route and
smaller lesions in rabbits at the site of intradermal
inoculation (Shida et al., 1988). The HA locus was used
for the insertion of foreign genes in the WR strain
20   (Shida et al., 1987), derivatives of the Lister strain
(Shida et al., 1988) and the Copenhagen strain (Guo et
al., 1989) of vaccinia virus. Recombinant HA⁻ vaccinia
virus expressing foreign genes have been shown to be
immunogenic (Guo et al., 1989; Itamura et al., 1990;
25   Shida et al., 1988; Shida et al., 1987) and protective
against challenge by the relevant pathogen (Guo et al.,
1989; Shida et al., 1987).

Cowpox virus (Brighton red strain) produces red
(hemorrhagic) pocks on the chorioallantoic membrane of
30   chicken eggs. Spontaneous deletions within the cowpox
genome generate mutants which produce white pocks (Pickup
et al., 1984). The hemorrhagic function ($\underline{u}$) maps to a 38
kDa protein encoded by an early gene (Pickup et al.,
1986). This gene, which has homology to serine protease
35   inhibitors, has been shown to inhibit the host
inflammatory response to cowpox virus (Palumbo et al.,
1989) and is an inhibitor of blood coagulation.

The u̲ gene is present in WR strain of vaccinia virus (Kotwal et al., 1989b). Mice inoculated with a WR vaccinia virus recombinant in which the u̲ region has been inactivated by insertion of a foreign gene produce higher antibody levels to the foreign gene product compared to mice inoculated with a similar recombinant vaccinia virus in which the u̲ gene is intact (Zhou et al., 1990). The u̲ region is present in a defective nonfunctional form in Copenhagen strain of vaccinia virus (open reading frames B13 and B14 by the terminology reported in Goebel et al., 1990a,b).

Cowpox virus is localized in infected cells in cytoplasmic A type inclusion bodies (ATI) (Kato et al., 1959). The function of ATI is thought to be the protection of cowpox virus virions during dissemination from animal to animal (Bergoin et al., 1971). The ATI region of the cowpox genome encodes a 160 kDa protein which forms the matrix of the ATI bodies (Funahashi et al., 1988; Patel et al., 1987). Vaccinia virus, though containing a homologous region in its genome, generally does not produce ATI. In WR strain of vaccinia, the ATI region of the genome is translated as a 94 kDa protein (Patel et al., 1988). In Copenhagen strain of vaccinia virus, most of the DNA sequences corresponding to the ATI region are deleted, with the remaining 3' end of the region fused with sequences upstream from the ATI region to form open reading frame (ORF) A26L (Goebel et al., 1990a,b).

A variety of spontaneous (Altenburger et al., 1989; Drillien et al., 1981; Lai et al., 1989; Moss et al., 1981; Paez et al., 1985; Panicali et al., 1981) and engineered (Perkus et al., 1991; Perkus et al., 1989; Perkus et al., 1986) deletions have been reported near the left end of the vaccinia virus genome. A WR strain of vaccinia virus with a 10 kb spontaneous deletion (Moss et al., 1981; Panicali et al., 1981) was shown to be attenuated by intracranial inoculation in mice (Buller et

al., 1985). This deletion was later shown to include 17
potential ORFs (Kotwal et al., 1988b). Specific genes
within the deleted region include the virokine N1L and a
35 kDa protein (C3L, by the terminology reported in
5   Goebel et al., 1990a,b). Insertional inactivation of N1L
reduces virulence by intracranial inoculation for both
normal and nude mice (Kotwal et al., 1989a). The 35 kDa
protein is secreted like N1L into the medium of vaccinia
virus infected cells. The protein contains homology to
10 the family of complement control proteins, particularly
the complement 4B binding protein (C4bp) (Kotwal et al.,
1988a). Like the cellular C4bp, the vaccinia 35 kDa
protein binds the fourth component of complement and
inhibits the classical complement cascade (Kotwal et al.,
15 1990). Thus the vaccinia 35 kDa protein appears to be
involved in aiding the virus in evading host defense
mechanisms.

         The left end of the vaccinia genome includes
two genes which have been identified as host range genes,
20 K1L (Gillard et al., 1986) and C7L (Perkus et al., 1990).
Deletion of both of these genes reduces the ability of
vaccinia virus to grow on a variety of human cell lines
(Perkus et al., 1990).

         To develop a new vaccinia vaccine strain, NYVAC
25 (vP866), the Copenhagen vaccine strain of vaccinia virus
was modified by the deletion of six nonessential regions
of the genome encoding known or potential virulence
factors. The sequential deletions are detailed in U.S.
Patents Nos. 5,364,773 and 5,494,807. All designations
30 of vaccinia restriction fragments, open reading frames
and nucleotide positions are based on the terminology
reported in Goebel et al., 1990a,b.

         The deletion loci were also engineered as
recipient loci for the insertion of foreign genes.
35   The regions deleted in NYVAC are listed below.
Also listed are the abbreviations and open reading frame
designations for the deleted regions (Goebel et al.,

33

1990a,b) and the designation of the vaccinia recombinant
(vP) containing all deletions through the deletion
specified:

         (1) thymidine kinase gene (TK; J2R) vP410;

         (2) hemorrhagic region (u̱; B13R + B14R) vP553;

         (3) A type inclusion body region (ATI; A26L)
vP618;

         (4) hemagglutinin gene (HA; A56R) vP723;

         (5) host range gene region (C7L - K1L) vP804;
and

         (6) large subunit, ribonucleotide reductase
(I4L) vP866

         (NYVAC).

NYVAC is a genetically engineered vaccinia
virus strain that was generated by the specific deletion
of eighteen open reading frames encoding gene products
associated with virulence and host range. NYVAC is
highly attenuated by a number of criteria including i)
decreased virulence after intracerebral inoculation in
newborn mice, ii) inocuity in genetically ($\underline{nu}^+$/$\underline{nu}^+$) or
chemically (cyclophosphamide) immunocompromised mice,
iii) failure to cause disseminated infection in
immunocompromised mice, iv) lack of significant
induration and ulceration on rabbit skin, v) rapid
clearance from the site of inoculation, and vi) greatly
reduced replication competency on a number of tissue
culture cell lines including those of human origin.
Nevertheless, NYVAC based vectors induce excellent
responses to extrinsic immunogens and provided protective
immunity.

Two additional vaccine vector systems involve
the use of naturally host-restricted poxviruses, avipox
viruses. Both fowlpoxvirus (FPV) and canarypoxvirus
(CPV) have been engineered to express foreign gene
products. Fowlpox virus (FPV) is the prototypic virus of
the Avipox genus of the Poxvirus family. The virus
causes an economically important disease of poultry which

has been well controlled since the 1920's by the use of
live attenuated vaccines. Replication of the avipox
viruses is limited to avian species (Matthews, 1982) and
there are no reports in the literature of avipox virus
5   causing a productive infection in any non-avian species
including man. This host restriction provides an
inherent safety barrier to transmission of the virus to
other species and makes use of avipox virus based vaccine
vectors in veterinary and human applications an
10 attractive proposition.

FPV has been used advantageously as a vector
expressing antigens from poultry pathogens. The
hemagglutinin protein of a virulent avian influenza virus
was expressed in an FPV recombinant (Taylor et al.,
15 1988a). After inoculation of the recombinant into
chickens and turkeys, an immune response was induced
which was protective against either a homologous or a
heterologous virulent influenza virus challenge (Taylor
et al., 1988a). FPV recombinants expressing the surface
20 glycoproteins of Newcastle Disease Virus have also been
developed (Taylor et al., 1990; Edbauer et al., 1990).

Despite the host-restriction for replication of
FPV and CPV to avian systems, recombinants derived from
these viruses were found to express extrinsic proteins in
25 cells of nonavian origin. Further, such recombinant
viruses were shown to elicit immunological responses
directed towards the foreign gene product and where
appropriate were shown to afford protection from
challenge against the corresponding pathogen (Tartaglia
30 et al., 1993a,b; Taylor et al., 1992; 1991b; 1988b).

The ALVAC recombinants can be by the methods
detailed in Piccini et al. 1983; Perkus et.al. 1995,
e.g., recombination, which is novel and nonobvious with
respect the present invention as a novel and nonobvious
35 product results therefrom.

TROVAC refers to an attenuated fowlpox that was
a plaque-cloned isolate derived from the FP-1 vaccine

strain of fowlpoxvirus which is licensed for vaccination
of 1 day old chicks. TROVAC is a unimolar fowlpox virus
species.

ALVAC is an attenuated canarypox virus-based
vector that was a plaque-cloned derivative of the
licensed canarypox vaccine, Kanapox (Tartaglia et al.,
1992). ALVAC has some general properties which are the
same as some general properties of Kanapox. ALVAC is a
unimolar canarypox virus species.

ALVAC-based recombinant viruses expressing
extrinsic immunogens have also been demonstrated
efficacious as vaccine vectors (Tartaglia et al.,
1993a,b). This avipox vector is restricted to avian
species for productive replication. On human cell
cultures, canarypox virus replication is aborted early in
the viral replication cycle prior to viral DNA synthesis.
Nevertheless, when engineered to express extrinsic
immunogens, authentic expression and processing is
observed *in vitro* in mammalian cells and inoculation into
numerous mammalian species induces antibody and cellular
immune responses to the extrinsic immunogen and provides
protection against challenge with the cognate pathogen
(Taylor et al., 1992; Taylor et al., 1991).

Recent Phase I clinical trials in both Europe
and the United States of a ALVAC recombinants, e.g.,
canarypox/rabies glycoprotein recombinant (ALVAC-RG),
demonstrated that ALVAC vaccines are safe and well
tolerated and, for instance, induced protective levels of
rabies virus neutralizing antibody titers (Fries et al.,
1996; Pialoux et al., 1994; Cadoz et al., 1992).
Additionally, peripheral blood mononuclear cells (PBMCs)
derived from the ALVAC-RG vaccinates demonstrated
significant levels of lymphocyte proliferation when
stimulated with purified rabies virus (Fries et al.,
1996).

NYVAC, ALVAC and TROVAC have also been
recognized as unique among all poxviruses in that the

National Institutes of Health ("NIH") (U.S. Public Health
Service), Recombinant DNA Advisory Committee, which
issues guidelines for the physical containment of genetic
material such as viruses and vectors, i.e., guidelines
5   for safety procedures for the use of such viruses and
vectors which are based upon the pathogenicity of the
particular virus or vector, granted a reduction in
physical containment level: from BSL2 to BSL1. No other
poxvirus has a BSL1 physical containment level. Even the
10   Copenhagen strain of vaccinia virus - the common smallpox
vaccine - has a higher physical containment level;
namely, BSL2. Accordingly, the art has recognized that
ALVAC has a lower pathogenicity than other poxvirus.

ALVAC-based recombinant viruses have been shown
15   to stimulate *in vitro* specific CD8^{+} CTLs from human PBMCs
(Tartaglia et al., 1993a). Mice immunized with ALVAC
recombinants expressing various forms of the HIV-1
envelope glycoprotein generated both primary and memory
HIV specific CTL responses which could be recalled by a
20   second inoculation (Tartaglia et al., 1993a; Cox et al.,
1993). ALVAC-env recombinants (expressing the HIV-1
envelope glycoprotein) stimulated strong HIV-specific CTL
responses from peripheral blood mononuclear cells (PBMC)
of HIV-1 infected individuals (Tartaglia et al., 1993a;
25   Cox et al., 1993). Acutely infected autologous PBMC were
used as stimulator cells for the remaining PBMC. After
10 days incubation in the absence of exogenous IL-2, the
cells were evaluated for CTL activities. ALVAC-env
stimulated high levels of anti-HIV activities in mice.
30   These and similar studies (see USSN 08/417,210) show a
utility of ALVAC- based recombinants, especially with
respect to immunodeficiency viruses. In particular, the
highly attenuated character of ALVAC has been
demonstrated in both immunocompetent and immuno-
35   compromised animal models in such studies; and, the
safety of ALVAC-based recombinants has also been
demonstrated.

Thus, in the present invention, the canarypox virus-based ALVAC vector is preferred.

Clearly, based on the attenuation profiles of the ALVAC vectors and its demonstrated ability to elicit

5   both humoral and cellular immunological responses to extrinsic immunogens (Tartaglia et al., 1993a,b; Taylor et al., 1992) such recombinant viruses offer a distinct advantage over previously described vaccinia based recombinant viruses.

10   Perhaps more related to FIV (as felines are involved), an ALVAC-based recombinant virus expressing the FeLV (Subgroup A) env and gag gene products (ALVAC-FL; vCP97) was shown to afford complete protection of cats against an oronasal FeLV challenge exposure

15   (Tartaglia et al., 1993). Significantly, protection was afforded in the absence of detectable FeLV-specific serum neutralizing activity prior to challenge.

In certain embodiments of the present invention, Applicants have engineered several ALVAC-FIV

20   recombinants and assessed their ability to afford protection of cats against experimental FIV exposure. In summary, Applicants have demonstrated protection from homologous FIV challenge exposure by vaccination of cats with an ALVAC-FIV Gag-protease recombinant. Recombinants

25   expressing FIV Env alone or in combination with Gag-protease did not afford significant levels of protection. However, vaccination regimens consisting of priming with ALVAC-FIV env/gag-protease and boosting with an adjuvanted inactivated whole cell vaccine preparation

30   provided complete protection, demonstrating utility for the recombinants expressing Env alone or in combination with Gag-protease, despite these recombinants not *per se* affording significant levels of protection (and further, these recombinants can be used in other aspects of the

35   invention, e.g., to express products which can nonetheless be useful, for instance to obtain useful antibodies, or in kits, tests, assays and the like).

Interestingly, levels of FIV-specific humoral
responses measured by ELISA and western blot were not
necessarily predictive of protection. Furthermore, Env-
specific humoral responses were not associated with the
5   observed protection.

Furthermore, the data herein shows the efficacy
of recombinants of the present invention against
heterologous FIV challenge in cats, especially in a
prime/boost protocol involving an inventive recombinant
10   (e.g., an ALVAC-FIV recombinant) and an ICV.

Moreover, the data herein with respect to FIV
and cats is capable of extension to other lentiviruses,
retroviruses, and immunodeficiency viruses, e.g., e.g.,
EIAV, FIV, BIV, HIV, or SIV. Thus, knowledge in the art
15   of nucleic acid molecules encoding epitope(s) of interest
from these other viruses, e.g., Env, Gag, protease, can
be utilized for making and using recombinants expressing
epitope(s) of interest analogous to the exemplified FIV
data herein. More in particular, using the knowledge in
20   the art of nucleic acid molecules encoding Env, Gag, Pol,
or a portion of Pol, such as a portion including
protease, accessory functions/proteins, or epitope(s)
thereof, for other lentiviruses, retroviruses, and
immunodeficiency viruses, e.g., EIAV, FIV, BIV, HIV, or
25   SIV, and using the knowledge in the art of vector
systems, the skilled artisan can make vectors or
recombinants expressing Env, Gag and Pol or a portion of
Pol, or Gag and Pol or a portion of Pol, or Env, Gag and
protease, or Gag and protease, with optionally accessory
30   functions/proteins, or expressing epitope(s) thereof, of
these other viruses, and can use the vectors or
recombinants in an immunization regimen, such as a
prime/boost regimen, as herein exemplified with respect
to FIV, without any undue experimentation. Accordingly,
35   the invention encompasses vectors or recombinants of
lentiviruses, retroviruses and immunodeficiency viruses
in addition to FIV (as FIV is a model for other

lentiviruses, retroviruses and immunodeficiency viruses),
and methods of making and using those vectors or
recombinants.

The expression product generated by inventive
vectors or recombinants can also be isolated from
infected or transfected cells and used to inoculate hosts
in a subunit vaccine configuration (composition, or an
antigenic or immunological composition). The proteins
generated by the vectors or recombinants and antibodies
elicited therefrom can also be used in assays to detect
the presence or absence of a lentivirus, retrovirus or
immunodeficiency virus, e.g., FIV.

Accordingly, the invention comprehends
immunogens or epitope(s) of interest such as lentivirus,
retrovirus or immunodeficiency virus immunogen(s) or
epitope(s) of interest, e.g., EIAV, FIV, BIV, HIV, or SIV
immunogens or epitopes of interest. Indeed, the
invention comprehends immunogens or epitopes of interest
from lentiviruses, including but not limited to HIV-1,-2,
EIAV, BIV. All lentiviruses express functional homologs
of the FIV Env, Gag-protease. Techniques for
identifying, cloning and utilizing nucleic acid sequences
encoding these functional homologs are known in the art
and do not require any undue experimentation to practice
in the light of this disclosure.

With respect to the state-of-the-art, mention
is particularly made of: Gonda et al. (1990).
Development of bovine immunodeficiency-like virus as a
model of lentivirus disease. Dev. Biol. Stand. 72:97-
110; Garvey et al. (1990) Nucleotide sequence and genome
organization of biologically active bovine
immunodeficiency-like virus. Virology 175:391-409; Gonda
et al. (1987). Characterization and molecular cloning of
a bovine lentivirus related to human immunodeficiency
virus. Nature 330:388-391; Ball et al. (1988). EIAV
genomic organization: further characterization by
sequencing of purified glycoproteins and cDNA. Virology

<u>165</u>: 601-605; Kawakami et al. (1987) Nucleotide sequence analysis of equine infectious anemia virus proviral DNA. <u>Virology</u> <u>158</u>: 300-312; Yaniv et al. (1986) Molecular cloning and physical characterization of integrated

5   equine infectious anemia virus:molecular and immunologic evidence of it's close relationship to ovine and caprine lentiviruses. <u>Virology</u> <u>154</u>: 1-8; Stephens et al. (1986). Equine infectious anemia virus <u>gag</u> and <u>pol</u> genes: relatedness to visna and AIDS virus. <u>Science</u> <u>231</u>:589-594;

10 Chiu et al. (1985). Nucleotide evidence for relationship of AIDS retrovirus to lentiviruses. <u>Nature</u> <u>317</u>:366-368; as well as a number of reviews in <u>Retrovirus Biology and Human Disease</u>, Gallo, R.C. and Wong-Stall, F. eds. Marcel Dekker, Inc. New York, 1990.

15   Further, DNA encoding such immunogens or epitopes of interest from inventive vectors or recombinants can be administered through immunization using alternate appropriately engineered mammalian expression systems including but not limited to other

20 poxviruses, herpesviruses, adenoviruses, alphavirus-based strategies, and naked or formulated DNA-based immunogens. Techniques for engineering such recombinant subunits are known in the art.
           With respect to techniques for these

25 immunization vehicles and state-of-the-art knowledge mention is particularly made of: Hormaeche and Kahn, Perkus and Paoletti, Shiver et al. all in <u>Concepts in Vaccine Development</u>, Kaufman, S.H.E., ed., Walter deGruytes, New York, 1996, and vectors described in

30 <u>Viruses in Human Gene Therapy</u>, Vos, J.-M.H., ed, Chapman and Hall, Carolina Academic Press, New York, 1995, and in <u>Recombinant Vectors in Vaccine Development</u>, Brown, F., ed., Karger, New York, 1994.
           The invention still further provides an

35 antigenic, immunogenic, immunological or vaccine composition containing the recombinant virus or expression product thereof, and a acceptable carrier or

diluent. An immunological composition containing the
vector or recombinant virus (or an expression product
thereof) elicits an immunological response - local or
systemic. The response can, but need not be, protective.
An immunogenic composition containing the vector or
recombinant virus (or an expression product thereof)
likewise elicits a local or systemic immunological
response which can, but need not be, protective. An
antigenic composition similarly elicits a local or
systemic immunological response which can, but need not
be, protective. A vaccine composition elicits a local or
systemic protective response. Accordingly, the terms
"immunological composition", "antigenic composition" and
"immunogenic composition" include a "vaccine composition"
(as the three former terms can be protective
compositions). A protective response is understood to be
a response, such as a humoral and/or secretory and/or
cell-mediated response which confers an immunity, with
immunity understood to comprise the ability to resist or
overcome infection or to overcome infection more easily
as compared to a subject not administered the inventive
composition, or to better tolerate infection as compared
to a subject not administered the inventive composition,
e.g., increased resistance to infection.

As to epitopes of interest, one skilled in the
art can determine an epitope or immunodominant region of
a peptide or polypeptide and ergo the coding DNA therefor
from the knowledge of the amino acid and corresponding
DNA sequences of the peptide or polypeptide, as well as
from the nature of particular amino acids (e.g., size,
charge, etc.) and the codon dictionary, without undue
experimentation.

A general method for determining which portions
of a protein to use in an immunological composition
focuses on the size and sequence of the antigen of
interest. "In general, large proteins, because they have
more potential determinants are better antigens than

small ones. The more foreign an antigen, that is the
less similar to self configurations which induce
tolerance, the more effective it is in provoking an
immune response." Ivan Roitt, <u>Essential Immunology</u>,
5   1988.

         As to size: the skilled artisan can maximize
the size of the protein encoded by the DNA sequence to be
inserted into the mammalian vector (keeping in mind the
insertion limitations of the vector). To minimize the
10   DNA inserted while maximizing the size of the protein
expressed, the DNA sequence can exclude introns (regions
of a gene which are transcribed but which are
subsequently excised from the primary RNA transcript).

         At a minimum, the DNA sequence can code for a
15   peptide at least 8 or 9 amino acids long. This is the
minimum length that a peptide needs to be in order to
stimulate a CD4+ T cell response (which recognizes virus
infected cells or cancerous cells). A minimum peptide
length of 13 to 25 amino acids is useful to stimulate a
20   CD8+ T cell response (which recognizes special antigen
presenting cells which have engulfed the pathogen). See
Kendrew, *The Encyclopedia of Molecular Biology* (Blackwell
Science Ltd 1995). However, as these are minimum
lengths, these peptides are likely to generate an
25   immunological response, i.e., an antibody or T cell
response; but, for a protective response (as from a
vaccine composition), a longer peptide is preferred.

         With respect to the sequence, the DNA sequence
preferably encodes at least regions of the peptide that
30   generate an antibody response or a T cell response. One
method to determine T and B cell epitopes involves
epitope mapping. The protein of interest "is fragmented
into overlapping peptides with proteolytic enzymes. The
individual peptides are then tested for their ability to
35   bind to an antibody elicited by the native protein or to
induce T cell or B cell activation. This approach has
been particularly useful in mapping T-cell epitopes since

the T cell recognizes short linear peptides complexed with MHC molecules. The method is less effective for determining B-cell epitopes" since B cell epitopes are often not linear amino acid sequence but rather result

5 from the tertiary structure of the folded three dimensional protein. Janis Kuby, Immunology, pp. 79-80 (1992).

Another method for determining an epitope of interest is to choose the regions of the protein that are

10 hydrophilic. Hydrophilic residues are often on the surface of the protein and are therefore often the regions of the protein which are accessible to the antibody. Janis Kuby, Immunology, p. 81 (1992).

Yet another method for determining an epitope

15 of interest is to perform an X-ray crystallographic analysis of the antigen (full length)-antibody complex. Janis Kuby, Immunology, p. 80 (1992).

Still another method for choosing an epitope of interest which can generate a T cell response is to

20 identify from the protein sequence potential HLA anchor binding motifs which are peptide sequences which are known to be likely to bind to the MHC molecule.

The peptide which is a putative epitope of interest, to generate a T cell response, should be

25 presented in a MHC complex. The peptide preferably contains appropriate anchor motifs for binding to the MHC molecules, and should bind with high enough affinity to generate an immune response. Factors which can be considered are: the HLA type of the patient (vertebrate,

30 animal or human) expected to be immunized, the sequence of the protein, the presence of appropriate anchor motifs and the occurrence of the peptide sequence in other vital cells.

An immune response is generated, in general, as

35 follows: T cells recognize proteins only when the protein has been cleaved into smaller peptides and is presented in a complex called the "major histocompatibility complex

MHC" located on another cell's surface. There are two
classes of MHC complexes - class I and class II, and each
class is made up of many different alleles. Different
patients have different types of MHC complex alleles;
they are said to have a 'different HLA type.'

Class I MHC complexes are found on virtually
every cell and present peptides from proteins produced
inside the cell. Thus, Class I MHC complexes are useful
for killing cells which when infected by viruses or which
have become cancerous and as the result of expression of
an oncogene. T cells which have a protein called CD4 on
their surface, bind to the MHC class I cells and secrete
lymphokines. The lymphokines stimulate a response; cells
arrive and kill the viral infected cell.

Class II MHC complexes are found only on
antigen- presenting cells and are used to present
peptides from circulating pathogens which have been
endocytosed by the antigen- presenting cells. T cells
which have a protein called CD8 bind to the MHC class I
cells and kill the cell by exocytosis of lytic granules.

Some guidelines in determining whether a
protein contains epitopes of interest which will
stimulate a T cell response, include: Peptide length -
the peptide should be at least 8 or 9 amino acids long to
fit into the MHC class I complex and at least 13-25 amino
acids long to fit into a class II MCH complex. This
length is a minimum for the peptide to bind to the MHC
complex. It is preferred for the peptides to be longer
than these lengths because cells may cut the expressed
peptides. The peptide should contain an appropriate
anchor motif which will enable it to bind to the various
class I or class II molecules with high enough
specificity to generate an immune response (See Bocchia,
M. et al., Specific Binding of Leukemia Oncogene Fusion
Protein Peptides to HLA Class I Molecules, Blood 85:2680-
2684; Englehard, VH, Structure of peptides associated
with class I and class II MHC molecules Ann. Rev.

Immunol. 12:181 (1994)). This can be done, without undue
experimentation, by comparing the sequence of the protein
of interest with published structures of peptides
associated with the MHC molecules. Protein epitopes
5   recognized by T cell receptors are peptides generated by
enzymatic degradation of the protein molecule and are
presented on the cell surface in association with class I
or class II MHC molecules.

Further, the skilled artisan can ascertain an
10 epitope of interest by comparing the protein sequence
with sequences listed in the protein data base. Regions
of the protein which share little or no homology are
better choices for being an epitope of that protein and
are therefore useful in a vaccine or immunological
15 composition. Regions which share great homology with
widely found sequences present in vital cells should be
avoided.

Even further, another method is simply to
generate or express portions of a protein of interest,
20 generate monoclonal antibodies to those portions of the
protein of interest, and then ascertain whether those
antibodies inhibit growth *in vitro* of the pathogen from
which the from which the protein was derived. The
skilled artisan can use the other guidelines set forth in
25 this disclosure and in the art for generating or
expressing portions of a protein of interest for analysis
as to whether antibodies thereto inhibit growth *in vitro.*

For example, the skilled artisan can generate
portions of a protein of interest by: selecting 8 to 9 or
30 13 to 25 amino acid length portions of the protein,
selecting hydrophilic regions, selecting portions shown
to bind from X-ray data of the antigen (full length)-
antibody complex, selecting regions which differ in
sequence from other proteins, selecting potential HLA
35 anchor binding motifs, or any combination of these
methods or other methods known in the art.

Epitopes recognized by antibodies are expressed
on the surface of a protein. To determine the regions of
a protein most likely to stimulate an antibody response
one skilled in the art can preferably perform an epitope
5   map, using the general methods described above, or other
mapping methods known in the art.

As can be seen from the foregoing, without
undue experimentation, from this disclosure and the
knowledge in the art, the skilled artisan can ascertain
10   the amino acid and corresponding DNA sequence of a
lentivirus epitope of interest for obtaining a T cell, B
cell and/or antibody response. In addition, reference is
made to Gefter et al., U.S. Patent No. 5,019,384, issued
May 28, 1991, and the documents it cites, incorporated
15   herein by reference (Note especially the "Relevant
Literature" section of this patent, and column 13 of this
patent which discloses that: "A large number of epitopes
have been defined for a wide variety of organisms of
interest. Of particular interest are those epitopes to
20   which neutralizing antibodies are directed. Disclosures
of such epitopes are in many of the references cited in
the Relevant Literature section.")

The administration procedure for the inventive
vector or recombinant or expression product thereof,
25   compositions of the invention such as immunological,
antigenic or vaccine compositions or therapeutic
compositions can be via a parenteral route (intradermal,
intramuscular or subcutaneous). Such an administration
enables a systemic immune response. The administration
30   can be via a mucosal route, e.g., oral, nasal, genital,
etc. Such an administration enables a local immune
response.

More generally, the inventive antigenic,
immunological or vaccine compositions or therapeutic
35   compositions (compositions containing the vectors or
recombinants of the invention or expression products) can
be prepared in accordance with standard techniques well

known to those skilled in the pharmaceutical or
veterinary arts. Such compositions can be administered
in dosages and by techniques well known to those skilled
in the medical and/or veterinary arts taking into
5   consideration such factors as the breed or species, age,
sex, weight, and condition of the particular patient, and
the route of administration. The compositions can be
administered alone, or can be co-administered or
sequentially administered with other compositions of the
10   invention or with other immunological, antigenic or
vaccine or therapeutic compositions. Such other
compositions can include purified native antigens or
epitopes or antigens or epitopes from the expression by a
poxvirus recombinant or another vector system; and are
15   administered taking into account the aforementioned
factors.
        Examples of compositions of the invention
include liquid preparations for orifice, e.g., oral,
nasal, anal, genital, e.g., vaginal, etc., administration
20   such as suspensions, syrups or elixirs; and, preparations
for parenteral, subcutaneous, intradermal, intramuscular
or intravenous administration (e.g., injectable
administration) such as sterile suspensions or emulsions.
In such compositions the recombinant may be in admixture
25   with a suitable carrier, diluent, or excipient such as
sterile water, physiological saline, glucose or the like.
        Antigenic, immunological or vaccine
compositions typically can contain an adjuvant and an
amount of the recombinant or expression product to elicit
30   the desired response. In human applications, alum
(aluminum phosphate or aluminum hydroxide) is a typical
adjuvant. Saponin and its purified component Quil A,
Freund's complete adjuvant and other adjuvants are used
in research and veterinary applications. Chemically
35   defined preparations such as muramyl dipeptide,
monophosphoryl lipid A, phospholipid conjugates such as
those described by Goodman-Snitkoff et al., _J. Immunol._

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147:410-415 (1991) and incorporated by reference herein, encapsulation of the protein within a proteoliposome as described by Miller et al., J. Exp. Med. 176:1739-1744 (1992) and incorporated by reference herein, and

5   encapsulation of the protein in lipid vesicles such as Novasome™ lipid vesicles (Micro Vesicular Systems, Inc., Nashua, NH) can also be used.

The compositions of the invention may be packaged in a single dosage form for immunization by

10   parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or orifice administration, e.g., perlingual (i.e., oral), intragastric, mucosal including intraoral, intraanal, intravaginal, and the like administration. And again, the effective dosage and

15   route of administration are determined by the nature of the composition, by the nature of the expression product, by expression level if the vector or recombinant is directly used, and by known factors, such as breed or species, age, sex, weight, condition and nature of host,

20   as well as LD_{50} and other screening procedures which are known and do not require undue experimentation.

Dosages of expressed product can range from a few to a few hundred micrograms, e.g., 5 to 500 μg. The inventive vector or recombinant can be administered in

25   any suitable amount to achieve expression at these dosage levels. The inventive vector or recombinant can be administered to an animal or infected or transfected into cells in an amount of about at least $10^{3.5}$ pfu; thus, the inventive vector or recombinant is preferably

30   administered to an animal or infected or transfected into cells in at least about 10^4 pfu to about 10^6 pfu; however, as shown by the Examples below, animals can be administered at least about 10^8 pfu such that a more preferred amount for administration can be at least about

35   10^7 pfu to about 10^9 pfu. Other suitable carriers or diluents can be water or a buffered saline, with or without a preservative. The expression product or vector

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or recombinant may be lyophilized for resuspension at the time of administration or can be in solution.

The carrier may also be a polymeric delayed release system. Synthetic polymers are particularly useful in the formulation of a composition having controlled release. An early example of this was the polymerization of methyl methacrylate into spheres having diameters less than one micron to form so-called nano particles, reported by Kreuter, J., <u>Microcapsules and Nanoparticles in Medicine and Pharmacology</u>, (M. Donbrow, ed.) CRC Press, p. 125-148.

Microencapsulation has been applied to the injection of microencapsulated pharmaceuticals to give a controlled release. A number of factors contribute to the selection of a particular polymer for microencapsulation. The reproducibility of polymer synthesis and the microencapsulation process, the cost of the microencapsulation materials and process, the toxicological profile, the requirements for variable release kinetics and the physicochemical compatibility of the polymer and the antigens are all factors that must be considered. Examples of useful polymers are polycarbonates, polyesters, polyurethanes, polyorthoesters and polyamides, particularly those that are biodegradable.

A frequent choice of a carrier for pharmaceuticals and more recently for antigens is poly (d,1-lactide-co-glycolide) (PLGA). This is a biodegradable polyester that has a long history of medical use in erodible sutures, bone plates and other temporary prostheses where it has not exhibited any toxicity. A wide variety of pharmaceuticals including peptides and antigens have been formulated into PLGA microcapsules. A body of data has accumulated on the adaption of PLGA for the controlled release of antigen, for example, as reviewed by Eldridge, J.H., et al., <u>Current Topics in Microbiology and Immunology,</u> 1989,

146:59-66.   The entrapment of antigens in PLGA
microspheres of 1 to 10 microns in diameter has been
shown to have a remarkable adjuvant effect when
administered orally.   The PLGA microencapsulation process
5   uses a phase separation of a water-in-oil emulsion.   The
compound of interest is prepared as an aqueous solution
and the PLGA is dissolved in a suitable organic solvents
such as methylene chloride and ethyl acetate.   These two
immiscible solutions are co-emulsified by high-speed
10   stirring.   A non-solvent for the polymer is then added,
causing precipitation of the polymer around the aqueous
droplets to form embryonic microcapsules.   The
microcapsules are collected, and stabilized with one of
an assortment of agents (polyvinyl alcohol (PVA),
15   gelatin, alginates, polyvinylpyrrolidone (PVP), methyl
cellulose) and the solvent removed by either drying *in
vacuo* or solvent extraction.

        Thus, solid, including solid-containing-liquid,
liquid, and gel (including "gel caps") compositions are
20   envisioned.   Additionally, the inventive
vector or recombinant, and the expression products
therefrom can stimulate an immune or antibody response in
animals.   From those antibodies, by techniques well-known
in the art, monoclonal antibodies can be prepared and,
25   those monoclonal antibodies, can be employed in well
known antibody binding assays, diagnostic kits or tests
to determine the presence or absence of antigen(s) and
therefrom the presence or absence of the natural
causative agent of the antigen or, to determine whether
30   an immune response to that agent or to the antigen(s) has
simply been stimulated.

        Monoclonal antibodies are immunoglobulin
produced by hybridoma cells.   A monoclonal antibody
reacts with a single antigenic determinant and provides
35   greater specificity than a conventional, serum-derived
antibody.   Furthermore, screening a large number of
monoclonal antibodies makes it possible to select an

individual antibody with desired specificity, avidity and
isotype. Hybridoma cell lines provide a constant,
inexpensive source of chemically identical antibodies and
preparations of such antibodies can be easily
standardized. Methods for producing monoclonal
antibodies are well known to those of ordinary skill in
the art, e.g., Koprowski, H. et al., U.S. Pat. No.
4,196,265, issued Apr. 1, 1989, incorporated herein by
reference.

          Uses of monoclonal antibodies are known. One
such use is in diagnostic methods, e.g., David, G. and
Greene, H., U.S. Pat. No. 4,376,110, issued Mar. 8, 1983,
incorporated herein by reference.

          Monoclonal antibodies have also been used to
recover materials by immunoadsorption chromatography,
e.g. Milstein, C., 1980, Scientific American 243:66, 70,
incorporated herein by reference.

          Furthermore, the inventive vector or
recombinant or expression products therefrom can be used
to stimulate a response in cells *in vitro* or *ex vivo* for
subsequent reinfusion into a patient. If the patient is
seronegative, the reinfusion is to stimulate an immune
response, e.g., an immunological or antigenic response
such as active immunization. In a seropositive patient,
the reinfusion is to stimulate or boost the immune system
against the lentivirus, retrovirus, or immunodeficiency
virus, e.g., FIV.

          Accordingly, the inventive vector or
recombinant has several utilities: In antigenic,
immunological or vaccine compositions such as for
administration to seronegative animals or humans (or
patients, as veterinarians like to call animals, with
"patients" including humans as well). In therapeutic
compositions in seropositive animals or humans in need of
therapy to stimulate or boost the immune system against
the lentivirus, retrovirus, or immunodeficiency virus,
e.g., feline immunodeficiency virus. *In vitro* to produce

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antigens or immunogens or epitopes of interest, which can
be further used in antigenic, immunological or vaccine
compositions or in therapeutic compositions. To generate
antibodies (either by direct administration or by
5   administration of an expression product of the inventive
vectors or recombinants) which can be further used: in
diagnosis, tests or kits to ascertain the presence or
absence of antigens or epitopes in a sample such as sera,
for instance, to ascertain the presence or absence of the
10   lentivirus, retrovirus, or immunodeficiency virus, e.g.,
feline immunodeficiency virus, in a sample such as sera
or, to determine whether an immune response has elicited
to the lentivirus, retrovirus, or immunodeficiency virus,
e.g., FIV, or, to particular antigen(s) or epitope(s);
15   or, in immunoadsorption chromatography. To generate DNA
for use as hybridization probes or to prepare PCR primers
or for DNA immunization. And, the inventive vectors or
recombinants, expression products therefrom, and
immunogens, antigens, and epitopes from the inventive
20   vectors or recombinants can be used to generate
stimulated cells which can be further used (reinfused) to
stimulate an immune response (antigenic, or immunological
response; or active immunization) or, to boost or
stimulate the immune system (for instance, of an
25   immunocompromised or seropositive animal or human).
Other utilities also exist for embodiments of the
invention.

     A better understanding of the present invention
and of its many advantages will be had from the following
30   examples, given by way of illustration.

EXAMPLES

     DNA Cloning and Synthesis. Plasmids were
constructed, screened and grown by standard procedures
(Maniatis et al., 1982; Perkus et al., 1985; Piccini et
35   al., 1987). Restriction endonucleases were obtained from
Bethesda Research Laboratories, Gaithersburg, MD, New
England Biolabs, Beverly, MA; and Boehringer Mannheim

Biochemicals, Indianapolis, IN. Klenow fragment of *E. coli* polymerase was obtained from Boehringer Mannheim Biochemicals. BAL-31 exonuclease and phage T4 DNA ligase were obtained from New England Biolabs. The reagents
5   were used as specified by the various suppliers.

Synthetic oligodeoxyribonucleotides were prepared on a Biosearch 8750 or Applied Biosystems 380B DNA synthesizer as previously described (Perkus et al., 1989). DNA sequencing was performed by the dideoxy-chain
10 termination method (Sanger et al., 1977) using Sequenase (Tabor et al., 1987) as previously described (Guo et al., 1989). DNA amplification by polymerase chain reaction (PCR) for sequence verification (Engelke et al., 1988) was performed using custom synthesized oligonucleotide
15 primers and GeneAmp DNA amplification Reagent Kit (Perkin Elmer Cetus, Norwalk, CT) in an automated Perkin Elmer Cetus DNA Thermal Cycler. Excess DNA sequences were deleted from plasmids by restriction endonuclease digestion followed by limited digestion by BAL-31
20 exonuclease and mutagenesis (Mandecki, 1986) using synthetic oligonucleotides.

Cells, Virus, and Transfection. The origins and conditions of cultivation of the Copenhagen strain of vaccinia virus has been previously described (Guo et al.,
25 1989). Generation of recombinant virus by recombination, *in situ* hybridization of nitrocellulose filters and screening for B-galactosidase activity are as previously described (Piccini et al., 1987).

The origins and conditions of cultivation of
30 the Copenhagen strain of vaccinia virus and NYVAC and ALVAC has been previously described (Guo et al., 1989; Tartaglia et al., 1992, U.S. Patents Nos. 5,364,773 and 5,494,807). Generation of recombinant virus by recombination, *in situ* hybridization of nitrocellulose
35 filters and screening for B-galactosidase activity are as previously described (Panicali et al., 1982; Perkus et al., 1989).

The parental canarypox virus (Rentschler
strain) is a vaccinal strain for canaries. The ALVAC
vaccine strain, as discussed above, was obtained from a
wild type isolate and attenuated through more than 200
5   serial passages on chick embryo fibroblasts. A master
viral seed was subjected to four successive plaque
purifications under agar and one plaque clone was
amplified through five additional passages after which
the stock virus was used as the parental virus in *in*
10 *vitro* recombination tests. The plaque purified canarypox
isolate is designated ALVAC.

The strain of fowlpox virus (FPV) designated
FP-1 has been described previously (Taylor et al.,
1988a). It is an attenuated vaccine strain useful in
15 vaccination of day old chickens. The parental virus
strain Duvette was obtained in France as a fowlpox scab
from a chicken. The virus was attenuated by
approximately 50 serial passages in chicken embryonated
eggs followed by 25 passages on chicken embryo fibroblast
20 cells. The virus was subjected to four successive plaque
purifications. One plaque isolate was further amplified
in primary CEF cells and a stock virus, designated as
TROVAC, established.

NYVAC, ALVAC and TROVAC viral vectors and their
25 derivatives were propagated as described previously
(Piccini et al., 1987; Taylor et al., 1988a,b, U.S.
Patents Nos. 5,364,773 and 5,494,807). Vero cells and
chick embryo fibroblasts (CEF) were propagated as
described previously (Taylor et al., 1988a,b).

30 **EXAMPLE 1   -   Construction of ALVAC-FIV env**

The feline immunodeficiency virus (FIV) <u>env</u>
coding sequence in plasmid ptg6184 and FIV nucleotide
sequences were obtained from Rhone Merieux (Lyon,
France). The cDNA clone was derived from the
35 Villefranche strain of FIV. The FIV <u>env</u> nucleotide
sequence is shown in Figure 2 (SEQ ID NO:1).

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The FIV <u>env</u> coding sequence was placed under
control of the modified early/late vaccinia virus H6
promoter (Perkus, et al., 1989). The FIV <u>env</u> coding
sequence contains two T₅NT sequence motifs which may
5   provide for premature early transcription termination
(Yuen and Moss, 1987). The T₅NT sequences were modified,
without altering the predicted amino acid coding
sequence, by replacement with a PCR-derived fragment.
TTTTTAT between positions 2059 and 2065 in Figure 2 was
10   changed to TTCTTAT; TTTTTCT between positions 2110 and
2116 was changed to TTCTTCT.
        Two overlapping PCR fragments were derived from
the ptg6184 template, yielding a fragment with altered
T₅NT sequences. A 585bp PCR fragment was generated using
15   oligonucleotide primers MM040 (SEQ ID NO:9) (5'-
AAATTCTTATATACAGCTTTCGCTATGCAAGAATTAGGATGTAATCAAAATCAATTC
TTCT GCAAAATCCCTCCTGGGT-3') and MM042 (SEQ ID NO:10) (5'-
CCCATCGAGTGCGGCTAC-3'). MM040 primes toward the 3'-most
sequences of the <u>env</u> coding sequence (from position 2056,
20   Figure 2). MM042 primes from the <u>env</u> 3'-most sequences
toward the 5'-most sequences. A second PCR primed with
MM041 (SEQ ID NO:11)
(5'GCAGAAGAATTGATTTTGATTACATCCTAATTCTT
GCATAGCGAAAGCTGTATATAAGAATTTTTCCATAGCTTC-3') and MM043
25   (SEQ ID NO:12) (5'AAGTTCTGGCAACCCATC-3') generated a
187bp fragment. MM041 primes from position 2118 toward
the 5'-most sequences of <u>env</u> and MM043 primes toward the
3'-most sequences of the <u>env</u> coding sequence from
position 1931 (Figure 2). The two PCR products were
30   pooled, primed with MM043 and MM042, and digested with
<u>Sca</u>I at FIV coding sequence position 2020 in Figure 2 and
<u>Eco</u>RI 3' of the <u>env</u> coding sequence. The resultant 564bp
<u>Sca</u>I-<u>Eco</u>RI PCR fragment contains the 3'-most sequences of
the FIV <u>env</u> coding sequence with the altered T₅NT motifs.
35   Plasmid ptg6184 was digested with <u>Eco</u>RI and
partially digested with <u>Sca</u>I. This ptg6184 derived
fragment with the 3' FIV <u>env</u> deleted from <u>Sca</u>I (Figure 2

position 2020) through EcoRI 3' of the env coding
sequence was ligated to the 564bp ScaI-EcoRI PCR-derived
fragment (above). The resultant plasmid pMM120 contains
the FIV env with altered T₅NT motifs. The nucleotide

5   sequence was confirmed using standard procedures (Goebel
et al., 1990a). The 2.6kbp pMM120 PstI-EcoRI fragment,
containing the FIV env coding sequence, was inserted
between the pBS-SK (Stratagene, La Jolla, California)
PstI and EcoRI sites generating pMM122.

10   The modified early/late vaccinia virus H6
promoter (Perkus et al., 1989) was added to pMM122 by
overlapping the H6 translation initiation codon with the
FIV env translation initiation codon. A fragment
containing the H6 promoted 5'-most sequences of the env

15   coding sequence was generated by PCR using primers MM037
(SEQ ID NO:13) (5'
ATCATCCTGCAGAAGCTTCCCGGGTTCTTTATTCTATACTT-3'), MM038 (SEQ
ID NO:14) (5'-CTGCAAATCCTTCTGCCATTACGATACAAACTTAAC-3'),
MM065 (SEQ ID NO:15) (5'-

20   CGTTAAGTTTGTATCGTAATGGCAGAAGGATTTGCAGCC-3'), and MM036
(SEQ ID NO:16) (5'-CCTCTTGAATTTCGTTCC-3'). pMM108,
containing H6 promoter sequences, was used as template
for PCR with MM037 and MM038 creating a 166bp fragment
containing the H6 promoter and the 5'-most bp of the FIV

25   env coding sequence. pMM122 was used as template for PCR
with MM065 and MM036 to generate a 235bp fragment with
the 3' H6 promoter and 5' env end. The two PCR products
were pooled, primed with MM036 and MM037, and the
resultant fragment, containing the H6 promoter fused to

30   the 5' most bp of the FIV env coding sequence, was
digested with PstI and KpnI generating a 266bp fragment.
pMM122 was digested with PstI and partially digested with
KpnI to remove the 5'-most
sequences of the FIV env coding region and the 266bp

35   PstI-KpnI PCR-derived fragment described above was
inserted. The resultant plasmid pMM125 contains the FIV

<u>env</u> juxtaposed 3' to the vaccinia H6 promoter in pBS-SK
(Stratagene, La Jolla, California).

        Sequence analysis of pMM125 demonstrated
correct PCR construction of the H6 promoted FIV <u>env</u> 5'-
5   most sequences, but frameshift mutations were observed 3'
of the PCR insertion. The frameshifts were not observed
in pMM122. All pMM125 clones contained frameshifts.
These frameshifts were probably a result of the recently
described instability of <u>env</u> sequences in high copy
10 number plasmids (Wang and Mullins, 1995). Separate
pMM125 clones had different frameshifts. An H6 promoted
FIV <u>env</u>, without frameshifts, was constructed in the
following manner.

        Briefly, this is a summary of the following
15 detailed description of the construction of an H6
promoted FIV <u>env</u> without frameshifts. The H6 promoted
5'-most sequences of the FIV <u>env</u> coding sequence, which
did not contain frameshifts, from one pMM125 clone was
ligated to the remaining unframeshifted 3'-most bp of FIV
20 <u>env</u> end from another pMM125 clone. The first fragment
was from the <u>Sma</u>I site 5' of the H6 promoter through the
<u>Afl</u>II site in the FIV <u>env</u> coding sequence (Figure 1,
position 1707). The second fragment was from the same
<u>Afl</u>II site to the <u>Sma</u>I site 3' of the <u>env</u> coding
25 sequence. The ligation product was digested with <u>Sma</u>I,
liberating three fragments. One fragment contained two
5'-most sequences and another fragment contained two 3'-
most sequences. The third <u>Sma</u>I digestion product
containing the H6 promoted FIV <u>env</u> expression cassette
30 was isolated and inserted into a C6 vector, generating
pRW945. To eliminate the possibility of frameshifts,
pRW945 was not amplified in bacteria. Details of pRW945
construction follow.

        One pMM125 clone, pMM125#11, had the correct
35 sequence from <u>Sma</u>I 5' of the H6 promoter through the
<u>Afl</u>II site at position 1707 (Figure 2); another pMM125
clone, pMM125#10, had the correct sequence from the <u>Afl</u>II

site at position 1707 through SmaI 3' of the env coding
sequence. The 1.8kbp pMM125#11 SmaI-AflII fragment,
containing the H6 promoted 5'-most env sequences, was
ligated to the 0.9kbp pMM125#10 SmaI-partial AflII

5   fragment containing the 3' portion of env. The ligation
product was SmaI digested and the 2.7kbp fragment was
inserted into the C6 vector pMM117, yielding pRW945.

      The C6 insertion plasmid, pMM117, was
constructed in the following manner. A 3kbp ALVAC

10   HindIII clone was sequenced and an open reading frame was
defined. A PCR-derived fragment was used for replacement
of the open reading frame with restriction sites for DNA
insertions. The PCR-derived fragment was generated with
primers C6A1 (SEQ ID NO:17) (5'-

15   ATCATCGAGCTCGCGGCCGCCTATCAAAAGTCTTAATGAGTT-3'), C6B1 (SEQ
ID NO:18) (5'-
GAATTCCTCGAGCTGCAGCCCGGGTTTTTATAGCTAATTAGTCAT
TTTTTCGTAAGTAAGTATTTTTATTTAA-3'), C6C1 (SEQ ID NO:19)
(5'-

20   CCCGGGCTGCAGCTCGAGGAATTCTTTTTATTGATTAACTAGTCAAATGAGTATATA
TAATTGA AAAAGTAA-3') and C6D1 (SEQ ID NO:20) (5'-
GATGATGGTACCTTCATAAATACAAGTTTGATTAAACTTAAGTTG-3'). ALVAC
was used as template for PCR using oligonucleotides C6A1
and C6B1 generating a 380bp fragment. A second PCR

25   reaction used ALVAC template and primers C6C1 and C6D1 to
generate a 1155bp fragment. The PCR reaction products
were pooled and primed for a final PCR with C6A1 and C6D1
yielding a 1613bp fragment. The final PCR product was
digested with SacI and KpnI for insertion between the

30   SacI and KpnI sites of pBS-SK (Stratagene, La Jolla,
California). The resultant C6 insertion plasmid was
designated pC6L. The C6 insertion plasmid pMM117 was
constructed by adding the sequence
GGGGGATCCTTAATTAATTAGTTATTAGACAAGGTGAAAACGAAACTATTTGTAGCT

35   TAATTAATTAGCTGCAGGAATTC (SEQ ID NO:21) between the pC6L
(Fig. 4; SEQ ID NO:3) SmaI and EcoRI sites.

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The above described plasmid pRW945 contains the
H6 promoted FIV _env_ coding sequence, with altered T$_5$NT
motifs, in a C6 insertion plasmid. pRW945 was used in _in
vitro_ recombination experiments with ALVAC as the
5   rescuing virus to derive recombinant vCP242. Figure 5
shows the predicted nucleotide sequence of the insertion
in vCP242 (SEQ ID NO:4).

**EXAMPLE 2 -   Construction of Recombinant ALVAC
                 Expressing FIV gag, and pro**

10

The feline immunodeficiency virus (FIV) _gag_ and
pol coding sequences in plasmid ptg8133 and FIV _env_
coding sequence in plasmid ptg6184 were obtained from
Rhone Merieux (Lyon, France). The cDNA clones were
15   derived from the Villefranche strain of FIV. Figure 3
(SEQ ID NO:2) contains the nucleotide sequence for the
FIV _gag_ and _pol_ coding regions obtained from Rhone
Merieux.

The FIV _gag_ sequences encoding core antigens,
20   followed by the _pol_ sequences encoding a protease,
reverse transcriptase and integrase, were placed under
control of the early/intermediate vaccinia I3L promoter
(Schmitt, J. and Stunnenberg, H., 1988; Vos, J. and
Stunnenberg, H., 1988). The I3L promoter corresponds to
25   positions 65073 through 64971 in Goebel et al., 1990 a,b.
The _gag_ and _pol_ coding sequences were engineered in a
single transcription unit. The Gag and Pol open reading
frames (ORFS) differ and translation of the Pol ORF
results via a ribosomal frameshift mechanism (Morikawa
30   and Bishop, 1992) as it does normally in FIV-infected
cells.

PCR-derived fragments were used for
construction of the I3L promoted FIV _gag_/_pol_ cassette.
The PCR-derived fragments were also used to alter a T$_5$NT
35   sequence motif which may provide for premature early
transcription termination (Yuen and Moss, 1987). TTTTTAT
between positions 467 and 473 (Figure 3) was changed to

TTTTCAT, without altering the predicted amino acid coding
sequence. Manipulations to construct the I3L promoted
FIV _gag_/_pol_ expression cassette were performed in the
following manner.

5   PCR was performed with ptg8133, containing the
FIV _gag_/_pol_ coding sequences, as template and MM027 (SEQ
ID NO:22) (5'-CAAAAATGGTGTCCATTTTCATGGAAAAGGCAAGAGAAGGAC-
3') and MM028 (SEQ ID NO:23) (5'-CTGCTGCAGTAAAATAGG-3')
as primers to generate a 245bp fragment. MM027 primed

10 from position 452 (Fig. 3) toward the 3'-most sequences
containing a nucleotide change in the T$_5$NT sequence motif.
MM028 primes from position 697 downstream of a _Hind_III
site toward the _gag_ 5'-most sequences. The 245bp PCR-
derived fragment contains the FIV _gag_ coding sequence

15 from position 452 to position 697 with an altered T$_5$NT
motif.

           A second PCR using ptg8133 as template and
primers MM029 (SEQ ID NO:24) (5'-
CTTCTCTTGCCTTTTCCATGAAAATGGACACCATTTT

20 TGGGTC-3') and MM030 (SEQ ID NO:25) (5'-
CAATTATTTAGGTTTAATCATGGGGAATGGACAGGGGC-3') generated a
508bp fragment. MM029 primes from position 490 (Fig. 3)
toward the 5'-most sequences of the _gag_ coding sequence
and alters the T$_5$NT sequence motif. MM030 contains the

25 3'-most sequence of the I3L promoter and primes from the
gag initiation codon toward the 3'-most sequences of the
gag coding sequence. The 508bp PCR-derived fragment
contains the 3'-most I3L promoter and the FIV _gag_ coding
sequence with an altered T$_5$NT motif through position 490.

30   Plasmid template pMM100, containing the I3L
promoter sequences, was primed with MM031 (SEQ ID NO:26)
(5'-CGCCCCTGTCCATTCCCCATGATTAAACCTAAATAATTGTAC-3') and
MM032 (SEQ ID NO:27) (5'-
ATCATCGTCGACATCGATACATCATGCAGTGGTTAAAC-3') to generate a

35 137bp PCR-derived fragment. The MM031 5'-most sequences
contains the 5'-most bp of _gag_ followed by a sequence
which primes at the I3L promoter 3'-most sequences toward

the I3L promoter 5'-most sequences. MM032 has <u>Sal</u>I and <u>Cla</u>I sites followed by a sequence which primes from the I3L promoter 5'-most sequences toward the 3'-most sequences. The 137bp PCR-derived fragment contains the
5 I3L promoted FIV <u>gag</u> 5'-most 20bp.

The three PCR products were pooled and primed for PCR with MM032 and MM028. The resultant 814bp fragment was digested with <u>Hind</u>III and <u>Sal</u>I, generating a 726bp fragment containing the I3L promoted FIV <u>gag</u> 5'-
10 most sequences with an altered T_5NT motif.

ptg8133 was digested with <u>Sac</u>I and <u>Sal</u>I, to remove the 7.2kbp plasmid sequences, and the 4.7kbp fragment was isolated and partially digested with <u>Hind</u>III. The 4kbp ptg8133 <u>Sac</u>I-<u>Hind</u>III partial digestion
15 product, containing the FIV <u>gag</u> coding sequence from position 615 through the FIV <u>pol</u> coding sequence, was isolated.

<u>Sac</u>I-<u>Sal</u>I digested pBS-SK (Stratagene, La Jolla, California) was ligated with the 726bp <u>Hind</u>III-
20 <u>Sal</u>I PCR-derived fragment (above) and the 4kbp ptg8133 <u>Sac</u>I-<u>Hind</u>III fragment. The resultant plasmid pMM116 contains the I3L promoted FIV <u>gag</u>/<u>pol</u> expression cassette in pBS-SK.

The 4.7kbp pMM116 <u>Asp718</u>-<u>Ecl136II</u> fragment
25 containing I3L promoted FIV <u>gag</u>/<u>pol</u> coding regions was treated with Klenow, in the presence of 20mM dNTPs, and inserted into <u>Sma</u>I digested pMM117 to produce pMM121. pMM117 is the C6 insertion plasmid described above.

The 1.4kbp pMM121 <u>Eco</u>RI fragment, containing
30 the I3L promoted FIV <u>gag</u>/<u>pol</u> 5'-most region, was inserted into the pBS-SK (Stratagene, La Jolla, California) <u>Eco</u>RI site generating pMM123. A PCR-derived fragment was used to remove the coding sequences corresponding to the carboxy-end of Pol to achieve Gag-protease expression
35 only. The PCR-derived fragment introduced a termination codon following the protease coding sequence at position 1709 (Fig. 3). Manipulations to construct the I3L

promoted FIV gag and protease coding sequences were
performed in the following manner.

Template pMM121, containing the I3L promoted
FIV gag/pol coding sequences, was primed with MM063 (SEQ
5   ID NO:28) (5'-CAGGACATCTAGCAAGAC-3') and MM064 (SEQ ID
NO:29) (5'-GATGATCCCGGGATAAAAATTATTGAGCCATTACTAACCT-3')
to generate a 580bp PCR-derived fragment. MM063 primes
from position 1148 (Fig. 3) toward the 3'-most sequences.
MM064 primes from position 1709 toward the 5'-most
10   sequences. The 580bp PCR-derived fragment, containing
the FIV protease coding sequence with a stop codon at
position 1709 (Fig. 3), was digested with EcoRI and SmaI
yielding a 475bp fragment.

pMM123 was linearized at the SmaI site 3' of
15   the FIV insertion, followed by partial EcoRI digestion.
The 475bp SmaI-EcoRI PCR-derived fragment (above) was
inserted into the pMM123 SmaI-EcoRI partial digestion
product, with the EcoRI site digested at figure 3
position 1246. The resultant plasmid pMM127 contains the
20   FIV gag and protease coding sequences, followed by a stop
codon, in pBS-SK (Stratagene, La Jolla, California). The
nucleotide sequence of the PCR-derived fragment in pMM127
was confirmed using standard procedures (Goebel et al.,
1990a). A single bp deletion 3' of the FIV protease
25   coding sequence was observed. MM064 was designed to add
TTTTTAT after the FIV protease stop codon. One T in the
TTTTTAT sequence after the stop codon is missing from
pMM127, resulting in the sequence TTTTAT.

The 1.8kbp pMM127 BamHI-SmaI fragment,
30   containing the I3L promoted FIV gag and protease coding
sequences, was inserted into SmaI-BamHI partially
digested pMM117. The C6 insertion plasmid pMM117 is
described above. The BamHI partial digestion was used to
digest the BamHI site next to the SmaI site in pMM117.
35   The resultant plasmid, containing the I3L promoted FIV
gag and protease coding sequences in a C6 insertion
plasmid, was designated pMM129. Plasmid pMM129 was used

in *in vitro* recombination experiments with ALVAC as the
rescuing virus to derive recombinant vCP253. Figure 6
(SEQ ID NO:5) shows the predicted nucleotide sequence of
the I3L promoted FIV *gag*/protease expression cassette and
5   flanking regions in vCP253.

EXAMPLE 3 -   Construction of Recombinant ALVAC
                Expressing FIV env, gag, and pro

          Plasmid pMM125, containing the H6 promoted FIV
env with frameshift mutations, is described above. A
10 deliberate insertion, containing a frameshift, into the
FIV *env* coding sequence allowed stable maintenance of the
remainder of the H6 promoted FIV *env* construct in
bacteria. After bacterial amplification the insertion
was removed. Manipulations to construct the H6 promoted
15 FIV *env* coding sequence, with a deliberate frameshift
insertion, were performed in the following manner.

          pMM125#11 (described above) was modified by
insertion of a PCR-derived fragment which repaired the
spontaneous frameshift and introduced a deliberate
20 frameshift flanked by BstEII sites. The BstEII insertion
is at position 1920 (Fig. 2). The insertion introduces a
stop codon followed by a frameshift, NotI and HindIII
sites. There are no BstEII sites in pMM125. The PCR-
derived fragment also changes the A at position 1920 in
25 figure 2 to C. The A to C change does not alter the
predicted amino acid coding sequence, but the change does
introduce a BstEII site. pMM125#10 has a spontaneous
frameshift at position 1604 (Figure 2). pMM125#10 was
used as template for PCR with oligonucleotide primers
30 RW542 (SEQ ID NO:30) (5'-TATGAATTGTAATTGTAC-3') and RW545
(SEQ ID NO:31) (5'-
GTAGCATAAGGTTACCGCGGCCGCTAAGCTTAGGTTACCATCCCTATAGCAGTA-
3') to generate a 326bp fragment containing the BstEII
insertion. RW542 primes from position 1632 toward the
35 FIV *env* 3'-most sequences; RW545 primes from position
1919 toward the FIV *env* 5'-most sequences (Figure 2).
pMM125#10 was used as template for PCR with RW544 (SEQ ID

NO:32) (5'-
GTAGCATAAGGTAACCTAAGCTTAGCGGCCGCGGTAACCCAATACCACCAAGTTCTG
GC-3 ') and T3 (SEQ ID NO:33) (5'-ATTAACCCTCACTAAAG-3')
generating a 791bp fragment containing the BstEII

5 insertion and the FIV env coding sequence 3'-most
sequences. RW544 primes from position 1914 toward the
env 3'-most sequences. T3 primes in the pBS-SK plasmid,
downstream of the FIV env stop codon, toward the FIV env
5'-most sequences. The two PCR products were pooled,

10 primed with RW542 and T3, and the resultant 1.1Kbp
product was digested with EcoRI and partially digested
with AflII generating a 876bp fragment which was inserted
into the following pMM125#11 vector. The PCR-derived
fragment and pMM125#11 vector were digested with AflII at

15 position 1709 (Fig. 2). pMM125#11 was digested with
EcoRI and EcoRI to remove the FIV env coding sequence 3'-
most sequences which contained a spontaneous frameshift.
The 876bp EcoRI-AflII PCR-derived fragment was inserted
into the pMM125#11 EcoRI-AflII vector. The resultant

20 plasmid pMM134 contains the FIV env coding sequence
juxtaposed 3' to the H6 promoter in pBS-SK (Stratagene,
La Jolla, California). pMM134 also contains a deliberate
frameshift mutation inserted between two BstEII sites.
The entire H6 promoted FIV env sequence in pMM134,

25 including the BstEII insertion, was confirmed. As
expected, the BstEII insertion allowed stable maintenance
of the remainder of the H6 promoted FIV env construct.
        Once the H6 promoted FIV env coding sequence
from pMM134 is cloned into a poxvirus insertion plasmid,

30 the BstEII insertion should be removed to allow
expression of the full length FIV env coding sequence.
The BstEII insertion is removed by BstEII digestion,
followed by a dilute ligation reaction favoring
intramolecular ligation. The intramolecular ligation

35 product would contain the H6 promoted FIV env, without
the BstEII insertion. After the BstEII insertion is
removed, the H6 promoted FIV env coding sequence is not

expected to be stably maintained in bacteria and the
plasmid was not amplified in bacteria. After ligation,
the plasmid was digested with NotI. Ligation products
containing the BstEII insertion would be digested at the

5   NotI site within the BstEII insertion. NotI digestion
within the BstEII insertion would prevent the ability of
the plasmid to generate a viable recombinant poxvirus.
Full length FIV env, without the BstEII insertion, would
not be cleaved by NotI digestion; FIV env coding

10   sequences would remain intact.

EXAMPLE 4   -   Construction of The H6 Promoted
                 FIV env Coding Sequence In C6 With
                 The I3L Promoted FIV gag And
                 Protease Coding Sequences

15   Construction of pMM134 containing the H6
promoted FIV env coding sequence with a BstEII insertion
has been described above. The 2.7kbp H6 promoted FIV env
SmaI fragment from pMM134, with the BstEII insertion, was
cloned into the following pMM129 insertion plasmid.

20   pMM129, containing the I3L promoted FIV gag and
protease coding sequences in C6, has been described
above. The pMM129 SalI site 5' of the I3L promoter was
blunt ended with Klenow in the presence of 20mM dNTPs.
pMM138 was constructed by insertion of the 2.7kbp pMM134

25   SmaI fragment containing the H6 promoted FIV env coding
sequence, with the BstEII insertion, into the pMM129
blunt ended SalI site. The H6 promoted FIV env coding
sequence, in pMM138, is 5' of the I3L promoted FIV gag
and protease coding sequences; the FIV coding sequences

30   are transcribed in the same direction.

             The two BstEII sites in pMM138 surround the
insertion containing a frameshift. Digestion of pMM138
with BstEII, to remove the insertion, was followed by
ligation. The resultant plasmid pMM146 was not amplified

35   in bacteria. pMM146 was designed for NotI digestion
before in vitro recombination experiments; NotI digestion
served two purposes. First, any plasmid unintentionally

containing the <u>Bst</u>EII insertion would be digested with
<u>Not</u>I within the insertion and the donor plasmid would be
prevented from generating a viable ALVAC recombinant.
Second, <u>Not</u>I linearizes pMM146 within the pBS-SK backbone
5   for efficient generation of the intended ALVAC
recombinant. pMM146 was digested with <u>Not</u>I prior to use
in *in vitro* recombination experiments with ALVAC as the
rescuing virus to derive recombinant vCP255. Figure 7
(SEQ ID NO:6) shows the predicted nucleotide sequence of
10   the H6 promoted FIV <u>env</u>/I3L promoted FIV <u>gag</u>/protease
expression cassette and flanking regions in vCP255.
     EXAMPLE 5   -   Construction of Recombinant ALVAC
                   Expressing FIV 97TM, gag, and pro

15   The FIV envelope glycoprotein is composed of
two cleavage products, gp97 and gp40. The FIV env coding
sequence was modified, by replacing gp40 with the
transmembrane anchor domain from the FIV env coding
sequences, in the following FIV 97TM construct. FIV
20   97TM, containing gp97 followed by the transmembrane
anchor domain, was constructed in the following manner.
             A PCR-derived fragment PCR-FIV1 (242bp) was
synthesized using pMM125#10 (containing the previously
described FIV <u>env</u> with the correct sequence from <u>Afl</u>II
25   site to 3'-most sequences) as a template, and
oligonucleotides MW196 (SEQ ID NO:34) (5'-
ACTTGCCATCGTCATGGGGG-3') and MW195A (SEQ ID NO:35) (5'-
GATACCTCCCAATAGTCCCCTTTTCCTTCTAGGTTTATATTC-3') as
primers. PCR-derived fragment PCR-FIV2 (193bp) was
30   synthesized using pMM125#10 as a template, and
oligonucleotides MW194A (SEQ ID NO:36) (5'-
GAATATAAACCTAGAAGGAAAAGGGGACTATTGGGAGGTATC-3')and MW197
(SEQ ID NO:37) (5'-
ATCATCGAATTCATAAAAATCATTCTTCTCCTTCTACTTC-3') as primers.
35   PCR-derived fragment PCR-FIV3 (393bp) was synthesized
using PCR-derived fragments PCR-FIV1 and PCR-FIV2 as
templates, and oligonucleotides MW196 and MW197 as

primers. A complete AflII/EcoRI digest of PCR-FIV3 was
performed, and the 284bp fragment was isolated. This
fragment was ligated into the 4.8kb AflII/EcoRI fragment
of pMM125#11 (containing the previously described FIV env

5   with the correct sequence from 5'-most sequences to the
EcoRI site described above). The resultant plasmid,
pMAW103, contains H6 promoted FIV 97TM.

        A PstI site was added upstream of the H6
promoter in the following manner. PCR-derived fragment

10 PCR-FIV4 (359bp) was synthesized using pMAW103 as a
template, and oligonucleotides MW209 (SEQ ID NO:38) (5'-
ATCATCAAGCTTCTGCAGTTCTTTATTCTA
TACTTA-3') and MM036 (SEQ ID NO:16) (5'-
CCTCTTGAATTTCGTTCC-3') as primers. A complete

15 HindIII/NruI digest of PCR-FIV4 was performed, and the
110bp fragment was inserted into the 5.0kb HindIII/NruI
fragment of pMAW103, yielding plasmid pMAW103A. The
2126bp pMAW103A PstI fragment containing the H6 promoted
FIV 97TM was inserted into the PstI site of pMM117

20 (described above), yielding plasmid pMAW104.

        The 2852bp pMAW104 BamHI partial digestion
product, containing H6 promoted FIV 97TM, was inserted
into BamHI digested pMM129 (I3L promoted FIV gag and pro
in C6 described above). The resultant plasmid pMAW105

25 contains H6 promoted FIV 97TM and I3L promoted FIV gag
and pro in C6. Plasmid pMAW105 was used in in vitro
recombination experiments with ALVAC as the rescuing
virus to derive recombinant vCP329. Figure 8 (SEQ ID
NO:7) shows the predicted nucleotide sequence of the

30 insertion to make vCP329.

EXAMPLE 6   -   ALVAC FIV Recombinant Expression Analysis

        Expression of the appropriate FIV-specific gene
products encoded by the ALVAC recombinants vCP242,
vCP253, vCP255, and vCP329 was demonstrated in various

35 analyses. Expression analyses were performed using
either appropriate monoclonal antibodies or serum derived
from FIV seropositive cats. Either reagent worked

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equally well in confirming expression in ALVAC-FIV-
infected cells. Accordingly, without undue
experimentation, from seropositive individuals,
monoclonals can be derived for confirming expression.

5   vCP242 FIV Env expression was demonstrated by
ELISA (described below). vCP242 was positive for surface
expression in an immunofluorescence assay by FACS with an
FIV Env specific monoclonal antibody (obtained from
Rhone-Merieux, Lyon, France). vCP242 was positive by

10   immunoprecipitation using polyclonal serum from FIV
infected cats and two different monoclonal antibodies
(described below). Thus, without undue experimentation,
monoclonals from seropositive individuals can be derived
for confirming expression.

15   vCP253 was positive for internal expression of
Gag by FACS. vCP253 was positive by immunoprecipitation
for expression of the mature Gag p24. A dominant Gag
precursor was detected at 37kDa; additional signals,
representing Gag cleavage products, were obtained at

20   49kDa, 40kDa, and 32kDa.

           vCP255 surface expression for Env was positive
by FACS with an Env-specific monoclonal antibody
(described below). vCP255 internal expression of Gag was
demonstrated with a Gag-specific monoclonal antibody by

25   FACS. vCP255 was assayed by immunoprecipitation with
monoclonal antibodies to each gene product: Gag was
positive with signals at approximately 49kDa, 40kDa,
37kDa, and 24kDa; FIV Env expression was positive with
signals at 130kDa and 90kDa.

30   vCP329 expression of 97TM and gag were detected
by immunoprecipitation with pooled serum from FIV
infected cats.

           FACS ANALYSIS: vCP255 contains the feline
immunodeficiency virus (FIV) env, gag and protease coding

35   sequences in locus C6. pMM146 was used in *in vitro*
recombination experiments with ALVAC as the rescuing
virus to derive recombinant vCP255. vCP255 FIV-specific

gene product expression was assayed on a fluorescence
activated cell sorter (FACS). The FIV <u>Env</u> protein
product was assayed on the surface of vCP255 infected
cells. The FIV p24 product was assayed for using
5   internal expression analyses. The antisera used for FACS
analysis were:

1)   monoclonal anti-FIV <u>env</u>: 128F10 EP110592 from
     Rhone Merieux (1:200 dilution)

2)   monoclonal anti-FIV p24: pool 125A3, 314B5
10   EP072092 from Rhone Merieux (1:100 dilution)

3)   monoclonal anti-rabies G: 24-3F-10 021387 from
     C. Trimarchi, Griffin Laboratories, New York
     State Health Department (1:200 dilution)

4)   polyclonal goat anti-mouse IgG coupled to
15   fluorescein isothiocyanate (FITC) from
     Boehringer Mannheim, catalogue number 605240,
     lot number 24064 (1:100 dilution)

FACS ANALYSIS OF EXPRESSION ON CELL SURFACE: 1
x 10^7 HeLa-S3 cells (ATCC #CCL2.2) were infected with 5 x
20   10^7 PFU of vCP255 in minimum essential medium (S-MEM:
Gibco #380-2380AJ) supplemented with 10% fetal bovine
sera, 20mM Glutamine and 0.5% penicillin-streptomycin.
The infected cells were incubated at 37°C for 30 minutes
with occasional agitation. The cells were washed with
25   10mls S-MEM. After each wash the cells were pelleted at
1000 RPM for 5 minutes in a Beckman GPKR centrifuge. The
infected cell pellet was resuspended in 1ml S-MEM,
transferred to a 5ml Sarstadt tube and slowly rotated at
37°C overnight.
30   After overnight incubation, 100μl aliquots of
the infected cells were added to 5ml polypropylene tubes.
The cells were washed with 3mls of PBS-CMF (137mM NaCl,
2.7mM KCl, 1.5mM KH_2PO_4, and 8mM Na_2HPO_4; pH 7.4) which
included 0.2% NaN_3 and 0.2 % bovine serum albumen (BSA).
35   The cells were pelleted and the supernatant was
discarded. Specific antibody was added to one tube and

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nonspecific antibody (anti-rabies G) was added to a
second tube in the following manner.

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          100μl of antibody (previously preadsorbed with
HeLa Cells) diluted in PBS-CMF supplemented with 0.2% NaN$_3$
and 0.2% BSA was added to each cell pellet, and incubated
at 4°C for 30 minutes. The cells were washed and
pelleted twice in 3mls of PBS-CMF containing 0.2% NaN$_3$ and
0.2% BSA. 100μl of secondary FITC coupled antibody
(previously preadsorbed with HeLa Cells) diluted 1:50 in
PBS-CMF containing 0.2% NaN$_3$ and 0.2% BSA was added and
incubated at 4°C, in the dark, for 30 minutes. The cells
were washed and pelleted twice in 3mls of PBS-CMF
containing 0.2% NaN$_3$ and 0.2% BSA. The cell pellets were
resuspended in 1ml PBS-CMF, containing 0.2% NaN$_3$ and 0.2%
BSA, transferred to 5ml polystyrene tubes and assayed on
the FACS.

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          FACSCAN ANALYSIS OF INTERNAL EXPRESSION: 1
x 10^7 HeLa-S3 cells (ATCC# CCL2.2) were infected with 5 x
10^7 PFU of vCP255 in minimum essential medium (S-MEM:
Gibco #380-2380AJ) supplemented with 10% fetal bovine
serum, 20mM Glutamine and 0.5% penicillin-streptomycin.
The infected cells were incubated at 37°C for 30 minutes
with occasional agitation. The cells were washed with
10mls S-MEM. After each wash the cells were pelleted at
1000 RPM for 5 minutes in a Beckman GPKR centrifuge. The
infected cell pellet was resuspended in 1ml S-MEM,
transferred to a 5ml Sarstadt tube and slowly rotated at
37°C overnight.

30

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          After overnight incubation, 100μl aliquots of
the infected cells were added to 5ml polypropylene tubes.
The cells were washed with 3mls PBS-CMF which contained
0.2% NaN$_3$. 100μl of 4% paraformaldehyde (Polysciences
Inc. #00380) pH 7.4 in PBS-CMF containing 0.2% NaN$_3$ was
added to the cell pellet and incubated on ice for 10
minutes. Specific antibody was added to one tube and
nonspecific antibody (anti-rabies G) was added to a
second tube in the following manner.

The paraformaldehyde treated cells were washed
with 3mls PBS-CMF containing 0.2% NaN$_3$. Following the
wash, 100μl PBS-CMF containing 0.2% NaN$_3$, 1% saponin
(SIGMA S-7900) and 20% heat inactivated newborn calf
5   serum (Gibco #200-6010AJ) was added. The cells were
incubated on ice for 30 minutes and washed with 3mls PBS-
CMF which contained 0.2% NaN$_3$.

100μl of antibody (previously preadsorbed with
HeLa Cells) diluted in PBS-CMF supplemented with 0.1%
10   saponin and 20% heat inactivated newborn calf serum was
added to each cell pellet, and incubated at 4°C for 30
minutes. The cells were washed and pelleted twice in
3mls of PBS-CMF containing 0.2% NaN$_3$ and 0.1% saponin.

100μl of secondary antibody coupled to FITC
15   (previously preadsorbed with HeLa Cells) diluted 1:50 in
PBS-CMF containing 0.2% NaN$_3$ and 0.1% saponin and 20% heat
inactivated newborn calf serum was added and incubated at
4°C, in the dark, for 30 minutes. The cells were washed
and pelleted twice in 3mls of PBS-CMF containing 0.2% NaN$_3$
20   and 0.1% saponin. The cell pellets were resuspended in
1ml PBS-CMF containing 0.2% NaN$_3$, transferred to 5ml
polystyrene tubes and assayed on the FACS.

vCP255 FACS ANALYSIS: Antisera/HeLa
suspensions were assayed on a Becton Dickinson model FC
25   FACScan flow cytometer. Data was analyzed on Lysis II
Software (Becton Dickinson, UK). The antisera/HeLa
suspensions were excited with a 488 nm argon laser, and
FITC emission spectra was identified using FL-1 channel
detectors. Ungated data was collected on 10,000 cells.
30   Fluorescence emission spectra, obtained by FACS
analysis of ALVAC infected HeLa cells, demonstrated
background levels of rabies G and FIV-specific gene
products. Background levels of the rabies G glycoprotein
were obtained by FACS analysis of vCP255 infected HeLa
35   cells.

The fluorescence emission spectra of vCP255
infected HeLa cells, probed with FIV specific monoclonal

antibodies, demonstrated expression of the FIV-specific
gene products. The FIV p24 coding sequence product was
detected internally from vCP255 infected HeLa cells. The
FIV Env product was detected on the surface of vCP255
5 infected HeLa cells.

IMMUNOPRECIPITATION ANALYSIS: CEF or VERO
cells were infected at an m.o.i. of 10 pfu/cell with
ALVAC (the parental virus), vCP242, vCP253, vCP255 or
vCP329. Following an hour adsorption period, the
10 inoculum was removed and the cells were overlaid with
2mls of modified Eagle's medium (minus cysteine and
methionine) containing 2% dialyzed fetal bovine serum and
[^{35}S]-TRANSlabel (New England Nuclear, Boston, MA;
30uCi/ml). The lysates were harvested 18-24 hrs post-
15 infection by addition of 1ml 3X buffer A (450mM NaCl, 3%
NP-40, 30mM Tris (pH7.4), 3mMEDTA, 0.03% Na-azide, and
0.6 mg/ml PMSF) and analyzed for expression of FIV env
and gag gene products. The above described polyclonal
cat antisera or FIV-specific monoclonal antibodies were
20 used for immunoprecipitation analysis in the following
manner.

Lysates were incubated overnight at 4°C with
FIV-specific antisera-protein A-sepharose complexes. The
samples were washed 4X with 1X buffer A and 2X with a
25 LiCl$_2$/urea buffer (200mM LiCl, 2M urea, and 10mm Tris
pH8.0). Precipitated proteins were dissociated from the
immune complexes by addition of 2X Laemmli buffer (124mM
Tris (pH6.8), 4% SDS, 20% glycerol, 10% 2-
mercaptoethanol) and boiling for 5 minutes. Proteins
30 were fractionated on 10% SDS-polyacrylamide gels, fixed
in methanol, and treated with 1M Na-salicylate for
fluorography. Proteins of the appropriate size were
precipitated from the lysates derived from cells infected
with the ALVAC-FIV recombinants, but were not
35 precipitated from uninfected or ALVAC infected cells.
The results indicated appropriate expression of the FIV
gene products by the ALVAC-FIV recombinants.

ELISA ANALYSIS: Primary chick embryo
fibroblast (CEF) cells were infected with vCP219, vCP242,
or ALVAC. The infected cells were analyzed with the
following FIV-specific monoclonal antibodies (Rhone
Merieux, Lyon, France).

        FIV gag: 0126B4 (anti-P15)

                    314B5   (anti-P24)

                    125A3   (anti-p24)

      FIV env: 128F10

                    117E5

                    115G8

SERUM SAMPLES TESTED BY ELISA:

        1.   Serum from FIV-infected cats:

              Received from Rhone Merieux.

              Cats #34 and #103

        2.   Normal cat serum: Cat #1229 (Select Labs,
              Athens, GA).

        3.   Rabbit serum obtained from immunization
              with vCP65 (ALVAC-RG):

              Rabbit A039:   prebleed

                              week 14

Infected cell lysates were prepared in the
following manner. Roller bottles of CEF cells were
infected with ALVAC, vCP219, or vCP242 at an MOI of 5 PFU
per cell in serum-free medium. Each roller bottle was
harvested at 20 hours post infection, when the cells were
completely round but not detached. Harvest consisted of
pouring off the medium, washing once with PBS, and
scraping the cells in 3 ml of PBS supplemented with
aprotinin (3.6 T.I.U; Sigma #A-6279). The harvested
cells were sonicated for four minutes on ice, and then
centrifuged for 10 min at 1000xg. Supernates were
recovered and the protein concentration was approximately
7 mg/ml for each preparation.

A kinetic ELISA was performed in the following
manner. Serum samples (above) were assayed by a sandwich
kinetic ELISA for the detection of FIV env and gag gene

products. Microtiter plates were coated with the pooled
monoclonal antibodies, listed above, specific for either
FIV env or FIV gag, at 2 or 5 μg/well. Infected cell
lysates were applied at 0.2, 1, or 5 μg/well, for capture

5   by the monoclonals. Each serum sample was assayed in
triplicate at a dilution of 1:100. Antibody was detected
with a 1:200 dilution of horse radish peroxidase(HRP)-
conjugated anti-cat serum (Jackson Immuno Research cat#
102-035-003) or HRP-conjugated anti-rabbit serum (DAKO,

10   cat# P217), followed by HRP substrate, o-phenylenediamine
dihycrochloride(OPD). The optical densities at A_{450} were
read for 15 min and rates for each sample were calculated
as mOD per minute.

Results from these ELISAs clearly demonstrated

15   that FIV Env and Gag expression were detected with serum
from FIV infected cats, but not normal cat serum (data
not shown). Env was demonstrated with plates prepared
using Env-specific MAb and lysates derived from cells
infected with vCP242, and not with lysates from ALVAC or

20   vCP219 infected cells. Similarly, Gag was demonstrated
with plates prepared using Gag-specific MAb and lysates
from cells infected with vCP219, and not ALVAC or vCP242
infected cells.

TABLE 1.   DETECTION OF FIV ENV EXPRESSION BY KINETIC ELISA

CAT SERUM:[b]	ALVAC lysate[a]		env lysate		gag lysate	
	NCS	FIV	NCS	FIV	NCS	FIV
lysate conc (µg/well)	KELISA (mOD/min)					
0.2	1.3	5.2	1.2	5.1	1.2	3.5
1	1.7	5.0	1.4	11.3	1.7	4.9
5	2.2	5.4	1.6	22.0	2.0	5.0
RABBIT SERUM:[c]	PB	Wk 14	PB	Wk 14	PB	Wk 14
0.2	1.3	4.8	0.9	2.3	0.9	2.8
1	1.0	5.0	0.7	4.1	0.8	3.0
5	1.1	6.4	0.9	3.0	1.0	4.6

a   Cell lysates from CEF cells infected with ALVAC, ALVAC-FIV env, or ALVAC-FIV gag were applied at 0.2, 1, or 5 µg/well to wells previously coated with 2 µg/well of pooled FIV env-specific MAb.

b   Cat sera: normal cat (NCS), FIV-infected cats (FIV).

c   Rabbit sera: prebleed (PB) and week 14 serum from rabbits inoculated with vCP-65 (NYVAC-RG).

TABLE 2. DETECTION OF FIV GAG-SPECIFIC ANTIBODIES BY KINETIC ELISA

CAT SERUM[b]	ALVAC lysate[a]		env lysate		gag lysate	
	NCS	FIV	NCS	FIV	NCS	FIV
lysate conc (μg/ml)	KELISA (mOD/min)					
0.2	2.7	6.7	1.5	3.9	1.4	10.4
1	2.3	6.2	1.8	3.6	1.3	30.7
5	2.7	6.5	1.9	4.3	1.7	32.2
RABBIT SERUM[c]	PB	wk 14	PB	wk 14	PB	wk 14
0.2	1.3	5.1	1.0	4.0	1.3	4.4
1	1.1	6.0	1.2	4.0	1.2	4.1
5	1.2	6.6	1.0	4.3	1.0	4.4

a   Cell lysates from CEF cells infected with ALVAC, ALVAC-FIV env, or ALVAC-FIV gag were applied at 0.2, 1, or 5 μg/well to wells previously coated with 2 μg/well of pooled FIV gag-specific MAb.

b   Cat sera: normal cat (NCS), FIV-infected cats (FIV).

c   Rabbit sera: prebleed (PB) and week 14 serum from rabbits inoculated with vCP-65 (NYVAC-RG).

EXAMPLE 7   -   <u>Efficacy of ALVAC-FIV Recombinants In Cats</u>

<u>Grouping And Immunization</u>: A total of 36 SPF

animals purchased from Liberty (Waverly, NY), age 12

weeks, were divided into seven groups as follows:

GroupsA	GroupB	GroupC	GroupD	GroupE	GroupF	GroupG
QH4 F	QH5 F	QQ1 F	QQ2 M	QH2 M	QH3 M	QC5 F
PY1 M	PY3 M	QA5 F	PY5 F	PY2 M	PY4 M	QG4 F
OO1 F	QS4 F	QU2 F	QO2 F	QA4 F	QA6 F	QE4 M
QC1 M	QC3 M	QX3 M	QX4 M	QC4 M		
QU1 M	QG3 F	QI1 M	QI2 M	QG5 F		
QL2 F	QE2 M	QL3 F	QL4 M	QE3 F		

Immunizations were administered as follows:

<u>Group A (6 cats)</u>   Immunization

(Days)

Primary immunization: ALVAC-*env* (vCP242)   Day 0

Secondary immunization: ALVAC-*env*   Day 28

Tertiary immunization: ALVAC-*env*   Day 56

<u>Group B (6 cats)</u>

Primary immunization: ALVAC-*env* , *gag/pro*

(vCP255)   Day 0

Secondary immunization: ALVAC-*env, gag/pro*   Day 28

Tertiary immunization: ALVAC-*env, gag/pro*   Day 56

<u>Group C (6 cats)</u>

Primary immunization: ALVAC-*gag/pro*

(vCP253)   Day 0

Secondary immunization: ALVAC-*gag/pro*   Day 28

Tertiary immunization: ALVAC-*gag/pro*   Day 56

Group D (6 cats)

Primary immunization: ALVAC-*97TM gag/pro*
(vCP329)   Day 0
5 Secondary immunization: ALVAC-*97TM* gag/pro   Day 28
Tertiary immunization: ALVAC-*97TM* gag/pro   Day 56

Group E (6 cats/control)

10 Primary immunization: ALVAC (CPpp)   Day 0
Secondary immunization: ALVAC   Day 28
Tertiary immunization: ALVAC   Day 56

Group F (3 cats/boost)
15

Primary immunization: ALVAC-*env* , *gag/pro*
(vCP255)   Day 0
Secondary immunization: ALVAC-*env,* *gag/pro*   Day 28
Boost: Inactivated FIV cell vaccine (ICV)   Day 56
20

Group G (3 cats/control)

Primary immunization: ALVAC   Day 0
Secondary immunization: ALVAC   Day 28
25 Boost: Inactivated FIV cell vaccine   Day 56

All cats received $1X10^8$ PFU of the respective
ALVAC recombinant in 1 ml sterile PBS via the
intramuscular route. The ICV boost consisted of $2.5x10^7$
30 fixed allogenic FIV-Petaluma infected feline T-cells (Fl-
4 cell line), mixed with 250 μg muramyl dipeptide (Hosie
et al., 1995). The ICV boost was given subcutaneously.
Challenge: All cats were challenged via an
intraperitoneal (IP) administration 4 weeks following
35 final immunization with 50 CID_{50} of FIV-Petaluma (cell
free supernatant derived from PBMC cultures infected with
FIV Petaloma strain).

The following assays were performed to
determine the FIV-specific virological status of the
challenged animals. This provided a direct measurement
of the protective efficacy of the ALVAC-based FIV vaccine
5   candidates.

1) Virus Isolation: Detection of Infectious
FIV by RT Assay. Peripheral blood mononuclear cells
(PBMCs), bone marrow (BM) cells, and lymph node (LN)
cells were collected upon challenge for virus isolation
10 (Yamamoto et al., 1991, 1993; Okada et al., 1994). Virus
isolation was performed by monitoring reverse
transcriptase (RT) activity of culture supernatants.
Isolated cells were cultured in the presence of IL-2 for
4 weeks. One-ml aliquots by standard procedures for Mg^{++}-
15 dependent RT activity (specific for lentiviruses).

2) FIV-specific PCR. Proviral sequence
detection was performed on DNA extracted from PBMC, BM,
and LN cells. As a means of increasing the sensitivity,
four consensus primer sets were used to amplify either env-
20   or gag-specific coding regions, respectively (Yamamoto
et al., 1991; Okada et al., 1994).

Following the initial PCR amplification, 1/25th
of the product was re-amplified with the nested primer
pair.
25   The results of the virological assays for
samples pre- and post-challenge are presented in Tables 3
and 4. None of the cats demonstrated FIV viremia prior
to challenge assessed either by RT determination or by
the FIV-specific PCR analysis (Table 3). By 8 weeks
30 post-challenge 4 of the 6 cats immunized with three doses
of the ALVAC parental virus developed a persistent FIV-
specific viremia (Table 3). Infection of these cats was
also demonstrable by virus isolation and PCR in tissue
samples taken post-challenge and by apparent FIV-specific
35 seroconversion post-challenge (Table 4 and 5). No clear
indications of infection were observed in the other two
cats (QA4 and QE3) in the control group. Further, in

comparison to this control group, no significant
differences in efficacy were observed in groups of cats
receiving three inoculations (10^8 pfu/dose) of ALVAC-FIV
env (vCP242), ALVAC-FIV env/gag-pr (vCP255), or ALVAC-FIV

5   97TMG (vCP329).

Significantly, three administrations of ALVAC-
FIV gag-pr(vCP253) afforded complete protection against
FIV challenge exposure. Protection from infection was
clearly evident in six of six cats throughout the 29 week

10   post-challenge observation period by virus isolation and
FIV-specific PCR in the periphery and lymphoid tissue
(Table 3 and 4). Further, these cats also did not
seroconvert relative to FIV seroreactivity by Western
blot or ELISA (Table 4 and 5). To further substantiate

15   the efficacy of vCP253, cells (PBMCs, lymph node, and
bone marrow) from two animals in this group were
transferred to SPF kittens. These cats have thus far
tested negative by virus isolation (RT and PCR) and FIV-
specific Western blot, whereas an SPF cats receiving

20   similar cells from an infected control cat (Py2) clearly
was positive for infection by these criteria.

Collectively, these results show that the Gag-
pr is sufficient to protect against a lentivirus
challenge exposure. As shown in Table 6, these results

25   are indeed statistically significant. The results also
show that the presence of Env may actually interfere with
the establishment of a protective immune response.
Further, the data for the experimental arm where cats
received vCP255 (2x) followed by ICV immunogen

30   illustrated that any impairment of Env can be overcome by
such a prime/boost regimen (Table 3 and 4). Clearly the
priming activity contributed by vCP255 was useful for
protection, since the cats in the group receiving 2
administrations of ALVAC parental virus followed by ICV

35   were readily infected upon challenge exposure (Table 3
and 4).

In short, this data provides for the first time protection against FIV infection in cats using a subunit immunogen, including only the FIV Gag-pr. In fact, the presence of Env may have reduced efficacy.

5   The importance of such data is also apparent in general for lentivirus vaccine development. Protection using solely the Gag-pr provides several important elements to vaccine and diagnostic design. First, one can readily employ existing Env-based assays to 10 discriminate vaccinated versus infected individuals. Secondly, the Gag-pr appears less variable than the Env species between lentivirus isolates and thus may serve for provision of cross-protective responses.

TABLE 3

Virus isolation (Reverse Transcriptase assay and PCR on PBMC)

Vaccine	Cat no.	Pre		Post challenge							
		RT	PCR	4 weeks		8 week		12 weeks		17 weeks	
				RT	PCR	RT	PCT	RT	PCT	RT	PCT
ALVAC- Env	QH4	−	−	−	−	−	−	−	−	−	−
	PY1	−	−	+	+	+	+	+	+	+	+
	QO1	−	−	+	+	+	+	T	T	−	−
	QC1	−	−	−	−	−	−	−	−	−	−
	QU1	−	−	−	−	−	−	−	−	−	−
	QL2	−	−	+	+	+	+	+	+	+	+
ALVAC- Eng, gag/prot	QH5	−	−	−	−	−	−	−	−	−	−
	PY3	−	−	−	−	−	−	−	−	−	−
	QS4	−	−	+	+	+	+	+	+	+	+
	QC3	−	−	−	−	−	−	−	−	−	−
	QG3	−	−	−	−	−	−	−	−	−	−
	QE2	−	−	−	−	−	−	+	+	−	+
ALVAC- gag/prot	QQ1	−	−	−	−	−	−	−	−	−	−
	QA5	−	−	−	−	−	−	−	−	−	−
	QU2	−	−	+	−	−	+	−	−	−	−
	QX3	−	−	+	−	−	+	−	−	−	−
	QI1	−	−	−	−	−	+	−	−	−	−
	QL3	−	−	−	−	−	−	−	−	−	−
ALVAC- 97TM of gag/prot	QQ2	−	−	−	−	−	−	−	−	−	−
	PY5	−	−	−	−	−	−	−	−	−	
	QO2	−	−	+	+	+	+	T*	T*		
	QX4	−	−	+	+	+	+	+	+	−	−
	QI2	−	−	−	+	+	+	+	+	+	+
	QL4	−	−	−	−	−	−	−	−	−	−
ALVAC- control	QH2	−	+	+	+	+	+	T*			
	PY2	−	−	−	−	+	+	+	+	+	+
	QA4	−	−	−	−	−	−	−	−	−	−
	QC4	−	−	−	−	+	+	+	+	+	+
	QG5	−	+	+	+	+	+	T*			
	QE3	−	−	−	−	−	−	−	−	−	−
ALVAC- env, gag, prot & ICV	QH3	−	−	−	−	−	−	−	−	−	−
	PY4	−	−	−	−	−	−	−	−	−	−
	QA6	−	−	−	−	−	−	−	−	−	−
ALVAC- control & ICV	QC5	−	−	+	+	+	+	+	+	T*	
	QG4	−	−	+	+	+	+	+	+	T*	
	QE4	−	−	−	−	+	+	+	+	+	+

*T: Animal was euthanized.
ND: Not Determined

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TABLE 4

FINAL VIRUS ISOLATION ON PBMC and TISSUE SAMPLES

Vaccine	Cat no.	TISSUE SAMPLE										
		PBL RT	PCR	LN RT	PCR	BM RT	PCR	THY RT	PCR	WB	Elisa	Tissues taken at x weeks post-chall.
ALVAC-Env	QH4	–	"+"	–	"+"	–	"+"	–	–	–	–	27
	PY1	–	–	–	+	–	–	+	+	+	+	24
	QO1	+	+	+	+	+	+	ND	ND	+	+	10
	QC1	–	–	–	–	–	–	ND	ND	–	–	28
	QU1	–	–	–	–	–	–	ND	ND	–	–	28
	QL2	–	–	+	+	+	+	–	–	+	+	24
ALVAC-Env, gag/prot	QH5	–	–	–	–	–	–	–	–	ND	–	28
	PY3	–	–	–	–	–	–	ND	ND	–	–	27
	QS4	–	–	+	+	–	+	–	–	+	+	24
	QC3	–	–	–	–	–	–	–	–	–	–	28
	QG3	–	–	–	–	–	–	–	–	–	–	28
	QE2	–	"+"	–	+	–	+	–	"+"	+	+	28
ALVAC-gag/prot	QQ1	–	–	–	–	–	–	ND	ND	–	–	29
	QA5	–	–	–	–	–	–	ND	ND	–	–	29
	QU2	–	–	–	–	–	–	ND	ND	–	–	29
	QX3	–	–	–	–	–	–	ND	ND	–	–	29
	QI1	–	–	–	–	–	–	ND	ND	–	–	29
	QL3	–	–	–	–	–	–	ND	ND	–	–	29
ALVAC-97TM OF gag/prot	QQ2	–	–	–	+	–	–	–	–	–	–	27
	PY5	–	–	–	–	–	–	–	–	–	–	28
	QO2	+	+	+	+	+	+	ND	ND	+	+	10
	QX4	–	–	–	–	–	–	–	–	+	–/+	25
	QI2	–	–	–	–	–	–	–	–	+	–	25
	QL4	–	–	–	–	–	–	–	–	ND	–	27
ALVAC control	QH2	+	+	+	+	+	+	ND	ND	+	–/+	10
	PY2	+	+	–	–	–	+	ND	ND	+	–	39
	QA4	–	–	–	–	–	–	–	–	–	–	28
	QC4	+	+	+	+	+	+	+	+	+	+	26
	QG5	–	+	+	+	–	+	ND	ND	+	+	10
	QE3	–	–	–	–	–	–	–	–	–	–	28
ALVAC-Env/gag/prot & ICV	QH3	–	–	–	–	–	–	ND	ND	+	–	36
	PY4	–	–	–	–	–	–	ND	ND	+	–	36
	QA6	–	–	–	–	–	–	ND	ND	+	–	36
ALVAC-control & ICV	QC5	+	+	+	+	–	–	ND	ND	+	+	10
	QG4	–	+	+	+	–	+	ND	ND	+	+	10
	QE4	+	+	ND	ND	+	+	ND	ND	+	+	39

*T: Animal was euthanized.
ND: Not Determined
"+": Show only very faintly positive by PCR.
NOTE Westernblot: serum dilution 1:100
ELISA: serum dilution 1:200, Transmembrane peptide used: QELGCNQNQFFCK1

EXAMPLE 8   -   ALVAC-FIV Recombinants induce protective Immunity
Against Multiple Subtype FIV Challenge In Cats

5   MATERIALS AND METHODS

Animals: Specific pathogen free (SPF), were purchased from Liberty Research, Inc.

Vaccine preparation: Canarypoxvirus (ALVAC)-FIV recombinants were generated as described above (vCP255).

10   The ALVAC vCP255 vaccine was prepared from a serum free lysate of infected CEF. ALVAC vCP255 immunizations were given at 1×10^8 PFU intramuscularly. The inactivated cell vaccine (ICV) consisted of 2×10^8 paraformaldehyde inactivated Fl-4 cells (a feline lymphoid cell line

15   chronically infected with FIV Petaluma) mixed with $250 \mu g$ SAF/MDP adjuvant (Hoise et al., 1995) and was given subcutaneously.

Grouping and immunization protocol: The challenge study involved 6 cats; the ALVAC-

20   *env,gag/pro*/ICV immunized group (#PY4, #QH3, #QA6) which received the FIV Petaluma challenge described in Example 7 and a control group of three age matched SPF cats (#EJ2, #DH3, #GU5) which had received no immunizations prior to the FIV Bangston challenge. (See Tables 5).

25   *Challenge:* The second challenge inoculum consisted of 75 ID_{50} cell free FIV Bangston (subtype B) and was given 8 months after the initial FIV Petaluma challenge (See Example 7).

Immunogenicity monitoring: The induction of

30   FIV specific antibody responses were determined by Western blotting (immunoblot). Viral neutralizing antibody responses (VNA) were determined using previously described assays (Yamamoto et al., 1991).

Viral infectivity monitoring: Viral infection

35   was monitored by several methods. This included assessment of viral reverse transcriptase activity in PBMC, bone marrow and lymph node cells taken at various times post-challenge by previously described methods (Yamamoto et al., 1991). In addition, pro-viral DNA

40   (latent infection) was monitored by polymerase chain

reaction (PCR) using FIV-_env_ primers on DNA extracted
from PBMC, bone marrow and lymph node cells upon
culturing for RT activity Yamamoto et al., 1991; Okada et
al, 1994). Further, FIV infection was determined by

5   monitoring and comparing the character of FIV-specific
humoral responses and viral neutralizing (VN) antibody
responses in serum taken before and after challenge.

RESULTS AND DISCUSSION

The immunogenicity and the protective efficacy

10 of ALVAC prime/boost protocols was evaluated against
experimental challenge with a distinctly heterologous FIV
isolate (Bangston strain). First, the protective
efficacy of immunizing with ALVAC-_env,gag/pol_ alone was
compared to priming with ALVAC-_env,gag/pol_ followed by

15 boosting with inactivated FIV-infected cell vaccine
(ICV). All cats received a total of three immunizations
and were challenged 4 weeks after the final immunization
with cell free 50 ID_{50} of FIV Petaluma (See Example 7).
The FIV Petaluma isolate, like the FIV Villefranche

20 isolate used to generate the ALVAC-FIV recombinant
vaccine, is classified as a subtype A virus and differs
from FIV Villefranche 3% in the Env and 1% in the Gag
amino-acid coding region.

It was then evaluated whether the ALVAC-

25 _env,gag/pro_/ICV immunized cats (#QA6, #QH3, #PY4) which
resisted the FIV Petaluma challenge described in Example
7, could be protected from a second challenge with a
distinctly heterologous FIV isolate of another subtype.
The second challenge consisted of 75 ID_{50} cell free FIV

30 Bangston (PBMC derived) and was given at eight months
after the initial challenge without any intervening
booster. FIV Bangston is a subtype B isolate and differs
from FIV Petaluma (subtype A) by 21% in the envelope
glycoprotein amino acid coding region. Three age matched

35 SPF cats (#EJ2, #GU5, #DH3) served as control cats for
the FIV Bangston challenge. As presented in Table 7, all
control cats became readily infected upon challenge. In
contrast, ALVAC-_env,gag/pro_/ICV immunized cats #QH3 and
#QA6 remained virus negative as determined by virus

40 isolation (RT) and PCR of peripheral blood up to three

months post-challenge. Cat #PY4 remained virus negative
as determined by virus isolation (RT) of peripheral blood
but tested positive by PCR at three months post-
challenge. Nucleotide sequence analysis of the PCR
5   product revealed FIV Bangston specific sequences. Thus
ALVAC-*env,gag/pro*/ICV immunized cats were partially
protected from a heterologous subtype FIV challenge. It
is clear that these cats demonstrated, at least, a delay
in infection as all control cats became viremic by 6
10   weeks post-challenge and only one of three ALVAC-
env,gag/pol/ICV immunized cats became positive based on
PCR analysis at 12 weeks post-challenge. This shows a
potential utility for recombinants expressing Env.

             In summary, prime/boost protocols involving
15   priming with ALVAC recombinants followed by boosting with
inactivated FIV-infected cell vaccines can elicit
protective immunity against experimental challenge with
heterologous FIV strains. This immunity is long lasting
and also provides partial protection against distinctly
20   heterologous FIV-strains of other subtypes. The data
supports a role for cell mediated rather than viral
neutralizing antibody responses and FIV-specific antibody
responses. These findings are relevant not only to the
development of multiple subtype FIV-vaccines but also to
25   the development of effective multiple subtype HIV
vaccines (as well as multiple subtype vaccines for other
lentiviruses and other retroviruses) as new subtypes
continue to arise and existing subtypes increasingly
spread to new geographical areas.

30   A Fisher's exact test was performed. This is a
modification of the Chi square test. This test should be
used when comparing two sets of discontinuous, quantal
(all or none) data. The analysis was set up as follows:

	Vaccinated	Unvaccinated
35 Infected	A	B
Uninfected	C	D

        For a single tailed probability the P value is
calculated as:

P(probability)=(A+B)!(C+D)!(A+C)!(B+D)!/N!A!B!C!D!

Each group was compared to the ALVAC-control group (n=6) and to the ALVAC-control group +ALVAC-control&ICV group (n=9). A P value equal or less than 0.05 was considered significant.

Table 5: Viral neutralizing antibody titers upon immunization.

Vaccine	Cat ID#	VN titer			
		pre-	post-immunizations	post-challenge	
				3 mo.	12 mo.
Alvac-env	QU1	<5	<5	<5	
	PY1	<5	<5	>100	
Alvac-gag/prot	QX3	<5	<5	<5	<5
	QQ1	<5	<5	<5	<5
	QI1	<5	<5	<5	<5
	QL3	<5	<5	<5	<5
Alvac-env,gag/prot	QS4	<5	<5	>100	
	PY3	<5	<5	<5	
Alvac-env,gag/prot &ICV	QH3	<5	<5	5-20	<5
	QA3	<5	<5	5-20	<5
	PY4	<5	<5	5-20	5-20
ALVAC-control	QC4	<5	<5	>100	
	PY4	<5	<5	>100	ND[a]
	QA4	in prep			
	QE3	in prep			
ALVAC-control &ICV	QG4	<5	<5	>100	
	QC5	<5	<5	>100	
	QE4	<5	<5	>100	ND

[a] ND - Not Determined

Table 6   Statistical significance of efficacy data.

vaccine	viral status			
	vaccine group +/-	control group +/- (control)	P value (Single-tailed)	significant
Alvac-*env*	3/3	4/2	0.5	no
		7/2	0.28	no
Alvac-*gag/prot*	0/6	4/2	0.0303	yes
		7/2	0.00914	yes
Alvac-*env, gag/prot*	2/4	4/2	0.28	no
		7/2	0.118	no
Alvac-*97TMG*	3/3	4/2	0.5	no
		7/2	0.28	no
Alvac-*env,gag/prot* *IWC*	0/3 0/3	3/0 7/2	0.05 0.00914	yes yes
All groups combined	8/19	7/2	0.0158	yes

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Table 7   Parameters pre- and post-secondary challenge (FIV Bangston)

	pre 2nd challenge WB titer	VN pre 2nd challenge[a]		VN post 2nd challenge[b]		post 2nd challenge[b]		Protection Rate
		FIVpet titer	FIVbang titer	FIVpet titer	FIVbang titer	WB titer	virus status RT/PCR	
QA6	+(4-5)	<5	ND	>5	ND	+(3-4)	-/-	
QH3	+(4)	<5	ND	>5	ND	+(3)	-/-	2/3
PY4	+(4-5)	5-20	ND	>5	ND	+(3-4)	-/+	
GU5	0(<2)	<5	<5	ND	ND	+(4-5)	+/+	
DH3	0(<2)	<5	<5	ND	ND	+(5)	+/+	0/3
EJ2	0(<2)	<5	<5	ND	ND	+(4)	+/+	

a   Serum sample taken 8 months post challenge, at the day of 2nd challenge.

b   Serum taken 4 months post 2nd-challenge.

c   ND - Not Determined

EXAMPLE 9   -   <u>Generation of Additional NYVAC & TROVAC</u>
<u>Recombinants</u>

Using the strategies outlined above for
generating FIV coding DNA linked to a promoter, flanking

5   DNA for NYVAC and TROVAC for insertion into regions of
these vectors, analogous to embodiments in U.S. Patent
No. 5,494,807 and USSN 08/417,210, are employed to
generate NYVAC and TROVAC FIV recombinants. Analysis
demonstrates incorporation into the vectors of the

10   exogenous DNA and of expression thereof. Such additional
recombinants are useful in the same manner as the above-
described ALVAC embodiments.

EXAMPLE 10   -   Generation of Additional Lentivirus
<u>and Additional Vector System Recombinants</u>

15   Using the strategies analogous to those
outlined above for generating FIV coding DNA linked to a
promoter and the strategies for generating alternative
poxvirus, baculovirus, adenovirus, herpesvirus,
alphavirus, poliovirus, Epstein-Barr, bacterial, and DNA-

20   based systems in the documents cited herein and the
knowledge of coding DNA from lentiviruses, retroviruses
or immunodeficiency viruses, e.g., EIAV, FIV, BIV, HIV,
or SIV, from the documents cited herein, alternative
poxvirus, baculovirus, adenovirus, herpesvirus,

25   alphavirus, poliovirus, Epstein-Barr, bacterial, and DNA-
based recombinants containing and expressing DNA from
lentiviruses, retroviruses or immunodeficiency viruses,
e.g., EIAV, FIV, BIV, HIV, or SIV, such as Env, Gag and
protease and Gag and protease, recombinants are

30   generated. Analysis demonstrates incorporation into the
vectors of the exogenous DNA and of expression thereof.
Such additional recombinants are useful in the same
manner as the above-described ALVAC embodiments.

Having thus described in detail preferred

35   embodiments of the present invention, it is to be
understood that the invention defined by the appended
claims is not to be limited by particular details set

forth in the above description as many apparent
variations thereof are possible without departing from
the spirit or scope thereof.

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WHAT IS CLAIMED IS:

1.   A vector comprising exogenous DNA encoding at least one lentivirus epitope.

2.   The vector of claim 1 wherein vector is a virus.

3.   The vector of claim 2 wherein virus is a poxvirus, adenovirus, or herpesvirus.

4.   The vector of claim 3 wherein herpesvirus is a feline herpesvirus vector.

5.   The vector of claim 3 wherein poxvirus is a vaccinia virus.

6.   The vector of claim 5 wherein vaccinia virus is the NYVAC strain.

7.   The vector of claim 3 wherein poxvirus is a canarypox virus.

8.   The vector of claim 7 wherein the canarypox virus is the ALVAC strain, or is a Rentschler vaccine strain which was attenuated through more than 200 serial passages on chick embryo fibroblasts, a master seed therefrom was subjected to four successive plaque purifications under agar, from which a plaque clone was amplified through five additional passages.

9.   The vector of claim 3 wherein the poxvirus is a fowlpox virus.

10.   The vector of claim 9 wherein the fowlpox virus is the TROVAC strain.

11.   The vector of claim 1 wherein vector is a naked or formulated DNA plasmid.

12.   The vector of claim 1 wherein the lentivirus is HIV-1.

13.   The vector of claim 1 wherein the lentivirus is HIV-2.

14.   The vector of claim 1 wherein the lentivirus is BIV.

15.   The vector of claim 1 wherein the lentivirus is FIV.

16.   The vector of claim 1 wherein the
lentivirus is EIAV.

17.   The vector of claim 1 wherein the
lentivirus is Visna virus.

18.   The vector of claim 1 wherein the
lentivirus is caprine arthritis-encephalitis virus.

19.   The vector of any one of claims 1 to 18
wherein the DNA encodes all or part of Gag-Pol, or Gag-
protease or Env, Gag-Pol, or Env, Gag-protease.

20.   The vector of any one of claims 1 to 18
wherein the DNA encodes all or part of Gag-Pol or Gag-
protease.

21.   The vector of any one of claims 1 to 18
wherein the DNA encodes all or part of Env, Gag-Pol, or
Env, Gag-protease.

22.   The vector of claim 15 which is vCP242,
vCP253, vCP255, or vCP329.

23.   A method for treating an animal or human
in need of immunological treatment or of inducing an
immunological response in an animal or human comprising
administering to said animal or human a composition
comprising a vector as claimed in any one of claims 1 to
18 in admixture with a suitable carrier.

24.   A method for treating an animal or human
in need of immunological treatment or of inducing an
immunological response in an animal or human comprising
administering to said animal or human a composition
comprising a vector as claimed in claim 20 in admixture
with a suitable carrier.

25.   A method for treating an animal or human
in need of immunological treatment or of inducing an
immunological response in an animal or human comprising
administering to said animal or human a composition
comprising a vector as claimed in claim 21 in admixture
with a suitable carrier.

26.   The method of claim 23 further comprising
additionally administering a respective lentivirus

epitope or a respective inactivated lentivirus either
prior or subsequent to administering the composition,
wherein the method is a prime-boost regimen.

27.   The method of claim 24 further comprising
additionally administering a respective lentivirus
epitope or a respective inactivated lentivirus either
prior or subsequent to administering the composition,
wherein the method is a prime-boost regimen.

28.   The method of claim 25 further comprising
additionally administering a respective lentivirus
epitope or a respective inactivated lentivirus either
prior or subsequent to administering to composition,
wherein the method is a prime-boost regimen.

29.   A composition for inducing an
immunological response comprising a vector as claimed in
any one of claims 1 to 18 in admixture with a suitable
carrier.

30.   A composition for inducing an
immunological response comprising a vector as claimed in
claim 20 in admixture with a suitable carrier.

31.   A composition for inducing an
immunological response comprising a vector as claimed in
claim 21 in admixture with a suitable carrier.

32.   A method for expressing a gene product in
a cell cultured *in vitro* comprising introducing into the
cell a vector as claimed in any one of claims 1 to 18.

33.   A feline immunodeficiency virus antigen
prepared from *in vitro* expression of a virus as claimed
in any one of claims 15 or 22.

34.   An antibody elicited by *in vivo* expression
of an antigen from a vector as claimed in any one of
claims 1 to 18 or, by administration of a lentivirus
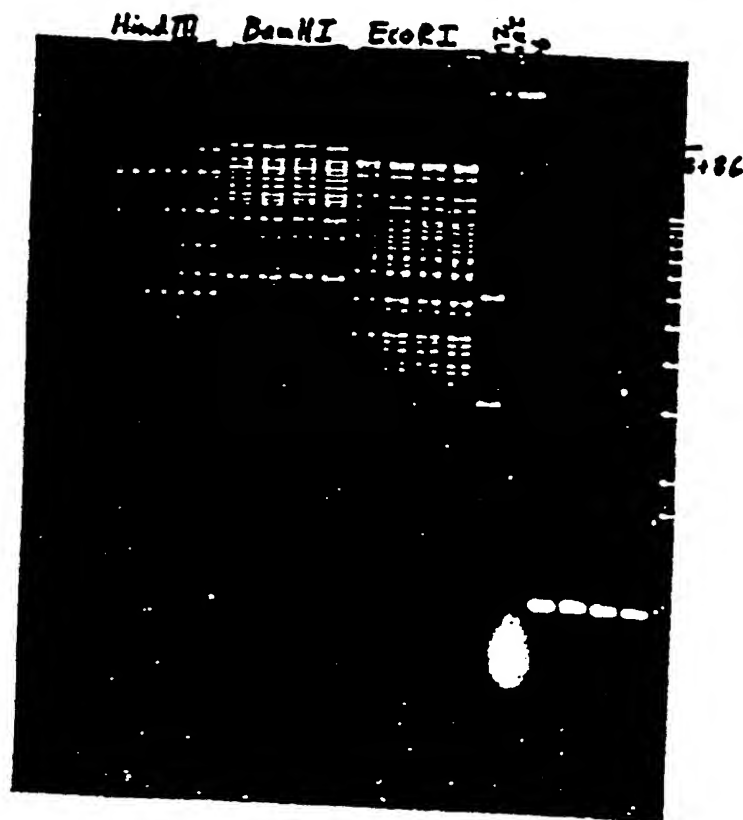associated antigen from *in vitro* expression of the
vector.

FIG. 1

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FIG. 2   Nucleotide sequence of FIV env from Rhone Merieux. The
FIV env start codon is at position 1 and the stop codon
is at position 2569. Plasmid ptg6184, containing the FIV
env coding sequence, was from Rhone Merieux. The FIV
env coding sequence in ptg6184 was sequenced and the
following differences with the sequence below were
observed: position 1218 T is G in ptg6184 changing phe to
leu; position 1220 G to A changes gly to glu; and
position 2201 C to A change ala to glu.

```
   1 ATGGCAGAAGGATTTGCAGCCAATAGACAATGGATAGGACCAGAAGAAGCTGAAGAGTTA
  61 TTAGATTTTGATATAGCAACACAAATGAGTGAAGAAGGACCACTAAATCCAGGAGTAAAC
 121 CCATTTAGGGTACCTGGAATAACAGAAAAAGAAAAGCAAAACTACTGTAACATATTACAA
 181 CCTAAGTTACAAGATCTAAGGAACGAAATTCAAGAGGTAAAACTGGAAGAAGGAAATGCA
 241 GGTAAGTTTAGAAGAGCAAGATTTTTAAGGTATTCTGATGAACAAGTATTGTCCCTGGTT
 301 CATGCGTTCATAGGATATTGTATATATTTAGGTAATCGAAATAAGTTAGGATCTTTAAGA
 361 CATGACATTGATATAGAAGCACCCCAAGAAGAGTGTTATAATAATAGAGAGAAGGGTACA
 421 ACTGACAATATAAAATATGGTAGCGATGTTGCCTAGGAACGGTGACTTTGTACCTGATT
 481 TTATTTATAGGATTAATAATATATTCACAGACAACCAACGCTCAGGTAGTATGGAGACTT
 541 CCACCATTAGTAGTCCCAGTAGAAGAATCAGAAATAATTTTTTGGGACTGTTGGGCACCA
 601 GAAGAACCCGCCTGTCAGGACTTTCTTGGGGCAATGATACATCTAAAAGCTAAGACAAAT
 661 ATAAGTATACGAGAGGGACCTACCTTGGGGAATTGGACTAGAGAAATATGGGCAACATTA
 721 TTCAAAAAGGCTACTAGACAATGTAGAAGAGGCAGAATATGGAAAAGATGGAATGAGACT
 781 ATAACAGGACCATCAGGATGTGCTAATAACACATGTTATAATGTTTCAGTAATAGTACCT
 841 GATTATCAGTGTTATTTAGATAGAGTAGATACTTGGTTACAAGGGAAAATAAATATATCA
 901 TTATGTCTAACAGGAGGAAAAATGTTGTACAATAAAGTTACAAAACAATTAAGCTATTGT
 961 ACAGACCCATTACAAATCCCACTGATCAATTATACATTTGGACCTAATCAAACATGTATG
1021 TGGAATACTTCACAAATTCAGGACCCTGAAATACCAAAATGTGGATGGTGGAATCAAATG
1081 GCCTATTATAACAGTTGTAAATGGGAAGAGGCAAAGGTAAAGTTTCATTGTCAAAGAACA
1141 CAGAGTCAGCCTGGATCATGGCGTAGAGCAATCTCGTCATGGAAACAAAGAAATAGATGG
1201 GAGTGGAGACCAGATTTTGGAAGTAAAAAGGTGAAAATATCTCTACAGTGCAATAGCACA
1261 AAAAACCTAACCTTTGCAATGAGAAGTTCAGGAGATTATGGAGAAGTAACGGGAGCTTGG
1321 ATAGAGTTTGGATGTCATAGAAATAAATCAAAACATCATTCTGAAGCAAGGTTTAGAATT
1381 AGATGTAGATGGAATGTAGGATCCGATACCTCGCTCATTGATACATGTGGAAACACTCGA
1441 GATGTTTCAGGTGCGAATCCTGTAGATTGTACCATGTATTCAAATAAAATGTACAATTGT
1501 TCTTTACAAAATGGGTTTACTATGAAGGTAGATGACCTTATTGTGCATTTCAATATGACA
1561 AAAGCTGTAGAAATGTATAATATTGCTGGAAATTGGTCTTGTACATCTGACTTGCCATCG
1621 TCATGGGGGTATATGAATTGTAATTGTACAAATAGTAGTAGTAGTTATAGTGGTACTAAA
1681 ATGGCATGTCCTAGCAATCGAGGCATCTTAAGGAATTGGTATAACCCAGTAGCAGGATTA
1741 CGACAATCCTTAGAACAGTATCAAGTTGTAAAACAACCAGATTACTTAGTGGTCCCAGAG
1801 GAAGTCATGGAATATAAACCTAGAAGGAAAAGGGCAGCTATTCATGTTATGTTGGCTCTT
1861 GCAACAGTATTATCTATTGTCGGTGCAGGGACGGGGGCTACTGCTATAGGGATGGTAACA
1921 CAATACCACCAAGTTCTGGCAACCCATCAAGAAGCTATAGAAAGGTGACTGAAGCCTTA
1981 AAGATAAACAACTTAAGATTAGTTACATTAGAGCATCAAGTACTAGTAATAGGATTAAAA
2041 GTAGAAGCTATGGAAAAATTTTTATATACAGCTTTCGCTATGCAAGAATTAGGATGTAAT
2101 CAAAATCAATTTTTCTGCAAAATCCCTCCTGGGTTGTGGACAAGGTATAATATGACTATA
2161 AATCAAACAATATGGAATCATGGAAATATAACTTTGGGGGCATGGTATAACCAAACAAAA
2221 GATTTACAACAAAAGTTTTATGAAATAATAATGGACATAGAACAAAATAATGTACAAGGG
2281 AAAACAGGGATACAACAATTACAAAAGTGGGAAGATTGGGTAGGATGGATGGGAAATATT
2341 CCACAATATTTAAAGGGACTATTGGGAGGTATCTTGGGAATAGGATTAGGAGTGTTATTA
2401 TTGATTTTATGTTTACCTACATTGGTTGATTGTATAAGAAATTGTATCCACAAGATACTA
2461 GGATACACAGTAATTGCAATGCCTGAAGTAGAAGGAGAAGAAATACAACCACAAATGGAA
2521 TTGAGGAGAAATGGTAGGCAATGTGGCATGTCTGAAAAAGAGGAGGAATGA
```

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FIG. 3   Nucleotide sequence of FIV gag/pol coding sequences from
Rhone Merieux. The gag start codon is at position 1 and
the gag stop codon is at position 1414. The ribosomal
frameshift site is near position 1255. The frameshift is
-1 in relation to the gag open reading frame. The
frameshift goes into the pol open reading frame. The pol
stop codon is at position 4614. Plasmid ptg8133 from
Rhone Merieux contains the FIV gag/pol coding sequences.
Part of ptg8133 has been sequenced and the CG at
positions 577-578 below is GC in ptg8133, changing the
codon from arg to ala.

```
1     ATGGGGAATGGACAGGGGCGAGATTGGAAAATGGCCATTAAGAGATGTAGTAATGTTGCT
61    GTAGGAGTAGGGGGGAAGAGTAAAAAATTTGGAGAAGGGAATTTCAGATGGGCCATTAGA
121   ATGGCTAATGTATCTACAGGACGAGAACCTGGTGATATACCAGAGACTTTAGATCAACTA
181   AGGTTGGTTATTTGCGATTTACAAGAAAGAAGAGAAAAATTTGGATCTAGCAAAGAAATT
241   GATATGGCAATTGTGACATTAAAAGTCTTTGCGGTAGCAGGACTTTTGAATATGACGGTG
301   TCTACTGCTGCTGCAGCTGAAAATATGTATTCTCAAATGGGATTAGACACTAGGCCATCT
361   ATGAAAGAAGCAGGTGGAAAAGAGGAAGGCCCTCCACAGGCATATCCTATTCAAACAGTA
421   AATGGAGTACCACAATATGTAGCACTTGACCCAAAAATGGTGTCCATTTTTATGGAAAAG
481   GCAAGAGAAGGACTAGGAGGGGAGGAAGTTCAACTATGGTTTACTGCCTTCTCTGCAAAT
541   TTAACACCTACTGACATGGCCACATTAATAATGGCCCGACCAGGGTGCGCTGCAGATAAA
601   GAAATATTGGATGAAAGCTTAAAGCAACTGACAGCAGAATATGATCGCACACATCCCCCT
661   GATGCTCCCAGACCATTACCCTATTTTACTGCAGCAGAAATTATGGGTATAGGATTAACT
721   CAAGAACAACAAGCAGAAGCAAGATTTGCACCAGCTAGGATGCAGTGTAGAGCATGGTAT
781   CTCGAGGCATTAGGAAAATTGGCTGCCATAAAAGCTAAGTCTCCTCGAGCTGTGCAGTTA
841   AGACAAGGAGCTAAGGAAGATTATTCATCCTTTATAGACAGATTGTTTGCCCAAATAGAT
901   CAAGAACAAAATACAGCTGAAGTTAAGTTATATATTAAAACAGTCATTAAGCATAGCTAAT
961   GCTAATGCAGACTGTAAAAAGGCAATGAGCCACCTTAAGCCAGAAAGTACCCTAGAAGAA
1021  AAGTTGAGAGCTTGTCAAGAAATAGGCTCACCAGGATATAAAATGCAACTCTTGGCAGAA
1081  GCTCTTACAAAAGTTCAAGTAGTGCAATCAAAAGGATCAGGACCAGTGTGTTTTAATTGT
1141  AAAAAACCAGGACATCTAGCAAGACAATGTAGAGAAGTGAAAAAATGTAATAAATGTGGA
1201  AAACCTGGTCATCTAGCTGCCAAATGTTGGCAAGGAAATAGAAAGAATTCGGGAAACTGG
1261  AAGGCGGGGCGAGCTGCAGCCCCAGTGAATCAAATGCAGCAAGCAGTAATGCCATCTGCA
1321  CCTCCAATGGAGGAGAAACTATTGGATTTATAAATTATAATAAAGTAGGTACGACTACAA
1381  CATTAGAAAAGAGGCCAGAAATACTTATATTTGTAAATGGATATCCTATAAAATTTTTAT
1441  TAGATACAGGAGCAGATATAACAATTTTAAATAGGAGAGATTTTCAAGTAAAAAATTCTA
1501  TAGAAAATGGAAGGCAAAATATGATTGGAGTAGGAGGAGGAAAGAGAGGAACAAATTATA
1561  TTAATGTACATTTAGAGATTAGAGATGAAAATTATAAGACACAATGTATATTTGGTAATG
1621  TTTGTGTCTTAGAAGATAACTCATTAATACAACCATTATTGGGGAGAGATAATATGATTA
1681  AATTCAATATTAGGTTAGTAATGGCTCAAATTTCTGATAAGATTCCAGTAGTAAAAGTAA
1741  AAATGAAGGATCCTAATAAAGGACCTCAAATAAACAATGGCCATTAACAAATGAAAAAA
1801  TTGAAGCCTTAACAGAAATAGTAGAAAGACTAGAAAGAGAAGGGAAAGTAAAAAGAGCAG
1861  ATCCAAATAATCCATGGAATACACCAGTATTTGCTATAAAAAAGAAAAGTGGAAAATGGA
1921  GAATGCTCATAGATTTTAGAGAATTAAACAAACTAACTGAGAAAGGAGCAGAGGTCCAGT
1981  TGGGACTACCTCATCCTGCTGGGTTACAAATAAAAAAACAAGTAACAGTATTAGATATAG
2041  GGGATGCATATTTCACCATTCCTCTTGATCCAGATTATGCTCCTTATACAGCATTTACTT
2101  TACCTAGGAAAAATAATGCGGGACCAGGAAGGAGATTTGTGTGGTGTAGTCTACCACAAG
2161  GCTGGATTTTAAGTCCATTGATATATCAAAGTACATTAGATAATATAATACAACCTTTTA
2221  TTAGACAAAATCCTCAATTAGATATTTACCAATATATGGATGACATTTATATAGGATCAA
2281  ATTTAAGTAAAAAGGAGCATAAAGAAAAGGTAGAAGAATTAAGAAAATTACTATTATGGT
2341  GGGGATTTGAAACTCCAGAAGATAAATTACAGGAAGAACCCCCATATACATGGATGGGTT
2401  ATGAATTACATCCATTAACATGGACAATACAACAGAAACAGTTAGACATTCCAGAACAGC
2461  CCACTCTAAATGAGTTGCAAAAATTAGCAGGAAAAATTAATTGGGCTAGCCAAGCTATTC
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FIG. 3 (cont'd)

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2521 CAGACTTGAGTATAAAAGCATTAACTAACATGATGAGAGGAAATCAAAACCTAAATTCAA
2581 CAAGACAATGGACTAAAGAAGCTCGACTGGAAGTACAAAAGGCAAAAAAGGCTATAGAAG
2641 AACAAGTACAACTAGGATACTATGACCCCAGTAAGGAGTTATATGCTAAATTAAGTTTGG
2701 TGGGACCACATCAAATAAGTTATCGAGTATATCAGAAGGATCAAGAAAAGATACTATGGT
2761 ATGGAAAAATGAGTAGACAAAAGAAAAAGGCAGAAAATACATGTGATATAGCCTTAAGAG
2821 CATGCTATAAGATAAGAGAAGAGTCTATTATAAGAATAGGAAAAGAACCAAGATATGAAA
2881 TACCTACTTCTAGAGAAGCCTGGGAATCAAATCTAATTAATTCACCATATCTTAAGGCCC
2941 CACCTCCTGAGGTAGAATATATCCATGCTGCTTTGAATATAAAGAGAGCGTTAAGTATGA
3001 TAAAAGATGCTCCAATACCAGGAGCAGAAACATGGTATATAGATGGAGGTAGAAAACTAG
3061 GAAAAGCAGCAAAAGCAGCCTATTGGACAGATACAGGAAAGTGGAAAGTGATGGAATTAG
3121 AAGGCAGTAATCAGAAGGCAGAAATACAAGCATTATTATTGGCATTAAAAGCAGGATCAG
3181 AGGAGATGAATATTATAACAGATTCACAATATGCTATAAATATTATTCTTCAACAACCAG
3241 ATATGATGGAGGGAATCTGGCAAGAAGTTTTAGAAGAATTGGAGAAGAAAACAGCAATAT
3301 TTATAGATTGGGTCCCAGGACATAAAGGTATTCCAGGAAATGAGGAAGTAGATAAGCTTT
3361 GTCAAACAATGATGATAATAGAAGGGGATGGGATATTAGACAAAAGGTCAGAAGATGCAG
3421 GATATGATTTATTAGCTGCAAAAGAAATACATTTATTGCCAGGAGAGGTAAAAGTAATAC
3481 CAACAGGGGTAAAGCTAATGCTGCCTAAAGGACATTGGGGATTAATAATCGGAAAAAGCT
3541 CGATGGGGAGTAAAGGATTGGATGTATTAGGAGGAGTAATAGATGAAGGATATCGAGGTG
3601 AAATTGGAGTAATAATGATTAATGTATCAAGAAAATCAATCACCTTAATGGAACGACAAA
3661 AGATAGCACAATTAATAATACTGCCTTGTAAACATGAAGTATTAGAACAAGGAAAAGTAG
3721 TAAGGGATTCAGAGAGAGGAGGCAATGGTTATGGGTCAACAGGAGTATTCTCCTCTTGGG
3781 TTGACAGAATTGAGGAAGCAGAAATAAATCATGAAAAATTTCACTCAGATCCACAGTACT
3841 TAAGGACTGAATTTAATTTACCTAAAATGGTAGCAGAAGAGATAAGACGAAAATGCCCAG
3901 TATGCAGAATCAGAGGAGAACAAGTGGGAGGACAATTGAAAATAGGGCCTGGTATCTGGC
3961 AAATGGATTGCACACACTTTGATGGCAAAATAATTCTTGTGGGTATACATGTGGAATCAG
4021 GATATATATGGGCACAAATAATTTCTCAAGAAACTGCTGACTGTACAGTTAAAGCTGTTT
4081 TACAATTGTTGAGTGCTCATAATGTTACTGAATTACAAACAGATAATGGACCAAATTTTA
4141 AAAATCAAAGATGGAAGGAGTACTCAATTACATGGGTGTGAAACATAAGTTTGGTATCC
4201 CAGGGAACCCACAGTCACAAGCATTAGTTGAAAATGTAAATCATACATTAAAAGTTTGGA
4261 TTCGGAAATTTTTGCCTGAAACAACCTCCTTGGATAATGCCTTATCTCTCGCTGTACATA
4321 GTCTCAATTTTAAAAGAAGAGGTAGGATAGGAGGGATGGCCCCTTATGAATTATTAGCAC
4381 AACAAGAATCCTTAAGAATACAAGATTATTTTTCTGCAATACCACAAAAATTGCAAGCAC
4441 AGTGGATTTATTATAAAGATCAAAAGATAAGAAATGGAAGGACCAATGAGAGTAGAAT
4501 ACTGGGGACAGGGATCAGTATTATTAAAGGATGAAGAGAAGGGATATTTTCTTATACCTA
4561 GGAGACACATAAGGAGAGTTCCAGAACCCTGCGCTCTTCCTGAAGGGGATGAGTGA
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FIG. 4   Sequence comprised in the C6 donor plasmid pC6L. Plasmid
         pC6L contains the C6 insertion sites SmaI (position 409)
         and EcoRI (position 425).

```
1    GAGCTCGCGGCCGCCTATCAAAAGTCTTAATGAGTTAGGTGTAGATAGTATAGATATTAC
61   TACAAAGGTATTCATATTTCCTATCAATTCTAAAGTAGATGATATTAATAACTCAAAGAT
121  GATGATAGTAGATAATAGATACGCTCATATAATGACTGCAAATTTGGACGGTTCACATTT
181  TAATCATCACGCGTTCATAAGTTTCAACTGCATAGATCAAAATCTCACTAAAAAGATAGC
241  CGATGTATTTGAGAGAGATTGGACATCTAACTACGCTAAAGAAATTACAGTTATAAATAA
301  TACATAATGGATTTTGTTATCATCAGTTATATTTAACATAAGTACAATAAAAAGTATTAA
361  ATAAAAATACTTACTTACGAAAAAATGACTAATTAGCTATAAAAACCCGGGCTGCAGCTC
421  GAGGAATTCTTTTTATTGATTAACTAGTCAAATGAGTATATATAATTGAAAAGTAAAAT
481  ATAAATCATATAATAATGAAACGAAATATCAGTAATAGACAGGAACTGGCAGATTCTTCT
541  TCTAATGAAGTAAGTACTGCTAAATCTCCAAAATTAGATAAAAATGATACAGCAAATACA
601  GCTTCATTCAACGAATTACCTTTTAATTTTTTCAGACACACCTTATTACAAACTAACTAA
661  GTCAGATGATGAGAAAGTAAATATAAATTTAACTTATGGGTATAATATAATAAAGATTCA
721  TGATATTAATAATTTACTTAACGATGTTAATAGACTTATTCCATCAACCCCTTCAAACCT
781  TTCTGGATATTATAAAATACCAGTTAATGATATTAAAATAGATTGTTTAAGAGATGTAAA
841  TAATTATTTGGAGGTAAAGGATATAAAATTAGTCTATCTTTCACATGGAAATGAATTACC
901  TAATATTAATAATTATGATAGGAATTTTTTAGGATTTACAGCTGTTATATGTATCAACAA
961  TACAGGCAGATCTATGGTTATGGTAAAACACTGTAACGGGAAGCAGCATTCTATGGTAAC
1021 TGGCCTATGTTTAATAGCCAGATCATTTTACTCTATAAACATTTTACCACAAATAATAGG
1081 ATCCTCTAGATATTTAATATTATATCTAACAACAACAAAAAAATTTAACGATGTATGGCC
1141 AGAAGTATTTTCTACTAATAAAGATAAAGATAGTCTATCTTATCTACAAGATATGAAAGA
1201 AGATAATCATTTAGTAGTAGCTACTAATATGGAAAGAAATGTATACAAAAACGTGGAAGC
1261 TTTTATATTAAATAGCATATTACTAGAAGATTTAAAATCTAGACTTAGTATAACAAAACA
1321 GTTAAATGCCAATATCGATTCTATATTTCATCATAACAGTAGTACATTAATCAGTGATAT
1381 ACTGAAACGATCTACAGACTCAACTATGCAAGGAATAAGCAATATGCCAATTATGTCTAA
1441 TATTTTAACTTTAGAACTAAAACGTTCTACCAATACTAAAAATAGGATACGTGATAGGCT
1501 GTTAAAAGCTGCAATAAATAGTAAGGATGTAGAAGAAATACTTTGTTCTATACCTTCGGA
1561 GGAAAGAACTTTAGAACAACTTAAGTTTAATCAAACTTGTATTTATGAAGGTACC
```

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FIG. 5   Predicted nucleotide sequence of the vCP242 insertion.
          The H6 promotor starts at position 55. The FIV env start
          codon is at position 179, and the FIV env stop codon is
          at position 2749. Positions 1 through 54 and positions
          2750 through 2879 flank the H6/FIV env expression
          cassette.

```
   1   TTAAATAAAAATACTTACTTACGAAAAATGACTAATTAGCTATAAAAACCCGGGTTCTTT
  61   ATTCTATACTTAAAAAGTGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTAAATTGAA
 121   AGCGAGAAATAATCATAAATTATTTCATTATCGCGATATCCGTTAAGTTTGTATCGTAAT
 181   GGCAGAAGGATTTGCAGCCAATAGACAATGGATAGGACCAGAAGAAGCTGAAGAGTTATT
 241   AGATTTTGATATAGCAACACAAATGAGTGAAGAAGGACCACTAAATCCAGGAGTAAACCC
 301   ATTTAGGGTACCTGGAATAACAGAAAAAGAAAAGCAAAACTACTGTAACATATTACAACC
 361   TAAGTTACAAGATCTAAGGAACGAAATTCAAGAGGTAAAACTGGAAGAAGGAAATGCAGG
 421   TAAGTTTAGAAGAGCAAGATTTTTAAGGTATTCTGATGAACAAGTATTGTCCCTGGTTCA
 481   TGCGTTCATAGGATATTGTATATATTTAGGTAATCGAAATAAGTTAGGATCTTTAAGACA
 541   TGACATTGATATAGAAGCACCCCAAGAAGAGTGTTATAATAATAGAGAGAAGGGTACAAC
 601   TGACAATATAAAATATGGTAGACGATGTTGCCTAGGAACGGTGACTTTGTACCTGATTTT
 661   ATTTATAGGATTAATAATATATTCACAGACAACCAACGCTCAGGTAGTATGGAGACTTCC
 721   ACCATTAGTAGTCCCAGTAGAAGAATCAGAAATAATTTTTTGGGACTGTTGGGCACCAGA
 781   AGAACCCGCCTGTCAGGACTTTCTTGGGGCAATGATACATCTAAAAGCTAAGACAAATAT
 841   AAGTATACGAGAGGGACCTACCTTGGGGAATTGGACTAGAGAAATATGGGCAACATTATT
 901   CAAAAAGGCTACTAGACAATGTAGAAGAGGCAGAATATGGAAAAGATGGAATGAGACTAT
 961   AACAGGACCATCAGGATGTGCTAATAACACATGTTATAATGTTTCAGTAATAGTACCTGA
1021   TTATCAGTGTTATTTAGATAGAGTAGATACTTGGTTACAAGGGAAAATAAATATATCATT
1081   ATGTCTAACAGGAGGAAAAATGTTGTACAATAAAGTTACAAAACAATTAAGCTATTGTAC
1141   AGACCCATTACAAATCCCACTGATCAATTATACATTTGGACCTAATCAAACATGTATGTG
1201   GAATACTTCACAAATTCAGGACCCTGAAATACCAAAATGTGGATGGTGGAATCAAATGGC
1261   CTATTATAACAGTTGTAAATGGGAAGAGGCAAAGGTAAAGTTTCATTGTCAAAGAACACA
1321   GAGTCAGCCTGGATCATGGCGTAGAGCAATCTCGTCATGGAAACAAAGAAATAGATGGGA
1381   GTGGAGACCAGATTTGGAAAGTAAAAAGGTGAAAATATCTCTACAGTGCAATAGCACAAA
1441   AAACCTAACCTTTGCAATGAGAAGTTCAGGAGATTATGGAGAAGTAACGGGAGCTTGGAT
1501   AGAGTTTGGATGTCATAGAAATAAATCAAAACATCATTCTGAAGCAAGGTTTAGAATTAG
1561   ATGTAGATGGAATGTAGGATCCGATACCTCGCTCATTGATACATGTGGAAACACTCGAGA
1621   TGTTTCAGGTGCGAATCCTGTAGATTGTACCATGTATTCAAATAAAATGTACAATTGTTC
1681   TTTACAAAATGGGTTTACTATGAAGGTAGATGACCTTATTGTGCATTTCAATATGACAAA
1741   AGCTGTAGAAATGTATAATATTGCTGGAAATTGGTCTTGTACATCTGACTTGCCATCGTC
1801   ATGGGGGTATATGAATTGTAATTGTACAAATAGTAGTAGTAGTTATAGTGGTACTAAAAT
1861   GGCATGTCCTAGCAATCGAGGCATCTTAAGGAATTGGTATAACCCAGTAGCAGGATTACG
1921   ACAATCCTTAGAACAGTATCAAGTTGTAAAACAACCAGATTACTTAGTGGTCCCAGAGGA
1981   AGTCATGGAATATAAACCTAGAAGGAAAAGGGCAGCTATTCATGTTATGTTGGCTCTTGC
2041   AACAGTATTATCTATTGTCGGTGCAGGGACGGGGGCTACTGCTATAGGGATGGTAACCCA
2101   ATACCACCAAGTTCTGGCAACCCATCAAGAAGCTATAGAAAAGGTGACTGAAGCCTTAAA
2161   GATAAACAACTTAAGATTAGTTACATTAGAGCATCAAGTACTAGTAATAGGATTAAAAGT
2221   AGAAGCTATGGAAAAATTCTTATATACAGCTTTCGCTATGCAAGAATTAGGATGTAATCA
2281   AAATCAATTCTTCTGCAAAATCCCTCCTGGGTTGTGGACAAGGTATAATATGACTATAAA
2341   TCAAACAATATGGAATCATGGAAATATAACTTTGGGGGAATGGTATAACCAAACAAAAGA
2401   TTTACAACAAAGTTTTATGAAATAATAATGGACATAGAACAAAATAATGTACAAGGGAA
2461   AACAGGGATACAACAATTACAAAAGTGGGAAGATTGGGTAGGATGGATGGGAAATATTCC
2521   ACAATATTTAAAGGGACTATTGGGAGGTATCTTGGGAATAGGATTAGGAGTGTTATTATT
2581   GATTTTATGTTTACCTACATTGGTTGATTGTATAAGAAATTGTATCCACAAGATACTAGG
2641   ATACACAGTAATTGCAATGCCTGAAGTAGAAGGAGAAGAAATACAACCACAAATGGAATT
2701   GAGGAGAAATGGTAGGCAATGTGGCATGTCTGAAAAGAGGAGGAATGATGAAGTATCTC
2761   AGAATTCCTGCAGCCCGGGGGATCCTTAATTAATTAGTTATTAGACAAGGTGAAAACGAA
2821   ACTATTTGTAGCTTAATTAATTAGCTGCAGGAATTCTTTTTATTGATTAACTAGTCAAA
```

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FIG. 6   Predicted nucleotide sequence of I3L promoted FIV
          gag/protease expression cassette and flanking regions in
          vCP253.   The I3L promoter begins at position 135.   The
          gag start codon is at position 235 and the protease stop
          codon is at position 1648.

```
   1    TTAATCAATAAAAAGAATTCCTGCAGCCCTGCAGCTAATTAATTAAGCTACAAATAGTTT
  61    CGTTTTCACCTTGTCTAATAACTAATTAATTAAGGATCCCCCGTACCGGGCCCCCCCTCG
 121    AGGTCGACATCGATACATCATGCAGTGGTTAAACAAAAACATTTTTATTCTCAAATGAGA
 181    TAAAGTGAAAATATATATCATTATATTACAAAGTACAATTATTTAGGTTTAATCATGGGG
 241    AATGGACAGGGGCGAGATTGGAAAATGGCCATTAAGAGATGTAGTAATGTTGCTGTAGGA
 301    GTAGGGGGGAAGAGTAAAAAATTTGGAGAAGGGAATTTCAGATGGGCCATTAGAATGGCT
 361    AATGTATCTACAGGACGAGAACCTGGTGATATACCAGAGACTTTAGATCAACTAAGGTTG
 421    GTTATTTGCGATTTACAAGAAAGAAGAGAAAAATTTGGATCTAGCAAAGAAATTGATATG
 481    GCAATTGTGACATTAAAAGTCTTTGCGGTAGCAGGACTTTTGAATATGACGGTGTCTACT
 541    GCTGCTGCAGCTGAAAATATGTATTCTCAAATGGGATTAGACACTAGGCCATCTATGAAA
 601    GAAGCAGGTGGAAAAGAGGAAGGCCCTCCACAGGCATATCCTATTCAAACAGTAAATGGA
 661    GTACCACAATATGTAGCACTTGACCCAAAAATGGTGTCCATTTTCATGGAAAAGGCAAGA
 721    GAAGGACTAGGAGGGGAGGAAGTTCAACTATGGTTTACTGCCTTCTCTGCAAATTTAACA
 781    CCTACTGACATGGCCACATTAATAATGGCCGCACCAGGGTGCGCTGCAGATAAAGAAATA
 841    TTGGATGAAAGCTTAAAGCAACTGACAGCAGAATATGATCGCACACATCCCCCTGATGCT
 901    CCCAGACCATTACCCTATTTTACTGCAGCAGAAATTATGGGTATAGGATTAACTCAAGAA
 961    CAACAAGCAGAAGCAAGATTTGCACCAGCTAGGATGCAGTGTAGAGCATGGTATCTCGAG
1021    GCATTAGGAAAATTGGCTGCCATAAAAGCTAAGTCTCCTCGAGCTGTGCAGTTAAGACAA
1081    GGAGCTAAGGAAGATTATTCATCCTTTATAGACAGATTGTTTGCCCAAATAGATCAAGAA
1141    CAAAATACAGCTGAAGTTAAGTTATATTTAAAACAGTCATTAAGCATAGCTAATGCTAAT
1201    GCAGACTGTAAAAAGGCAATGAGCCACCTTAAGCCAGAAAGTACCCTAGAAGAAAAGTTG
1261    AGAGCTTGTCAAGAAATAGGCTCACCAGGATATAAAATGCAACTCTTGGCAGAAGCTCTT
1321    ACAAAAGTTCAAGTAGTGCAATCAAAAGGATCAGGACCAGTGTGTTTTAATTGTAAAAAA
1381    CCAGGACATCTAGCAAGACAATGTAGAGAAGTGAAAAAATGTAATAAATGTGGAAAACCT
1441    GGTCATCTAGCTGCCAAATGTTGGCAAGGAAATAGAAAGAATTCGGGAAACTGGAAGGCG
1501    GGGCGAGCTGCAGCCCCAGTGAATCAAATGCAGCAAGCAGTAATGCCATCTGCACCTCCA
1561    ATGGAGGAGAAACTATTGGATTTATAAATTATAATAAAGTAGGTACGACTACAACATTAG
1621    AAAAGAGGCCAGAAATACTTATATTTGTAAATGGATATCCTATAAAATTTTTATTAGATA
1681    CAGGAGCAGATATAACAATTTTAAATAGGAGAGATTTTCAAGTAAAAAATTCTATAGAAA
1741    ATGGAAGGCAAAATATGATTGGAGTAGGAGGAGGAAAGAGAGGAACAAATTATATTAATG
1801    TACATTTAGAGATTAGAGATGAAAATTATAAGACACAATGTATATTTGGTAATGTTTGTG
1861    TCTTAGAAGATAACTCATTAATACAACCATTATTGGGGAGAGATAATATGATTAAATTCA
1921    ATATTAGGTTAGTAATGGCTCAATAATTTTATCCCGGGTTTTTATAGCTAATTAGTCATT
1981    TTTCGTAAGTAAGTATTTTTATTTAATACTTTTTATTGTACTTATGTTAAAT
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FIG. 7   Predicted nucleotide sequence of the H6 promoted FIV
           env/I3L promoted FIV gag/protease expression cassette and
           flanking regions in vCP255. The H6 promotor starts at
           position 129, the FIV env start codon is at position 253,
           and the FIV env stop codon is at position 2823. The I3L
           promotor starts at position 2830, the FIV gag start codon
           is at position 2930 and the FIV gag stop codon is at
           position 4282. The ribosomal frameshift site is near
           position 4184. The frameshift is -1 in relation to the
           gag open reading frame. The frameshift goes into the pol
           open reading frame. The stop codon for the protease gene
           is at position 4641.   Positions 1 through 128 and
           positions 4642 through 4727 flank the H6 FIV env/I3L FIV
           gag/protease expression cassette.

```
   1  TTAATCAATAAAAAGAATTCCTGCAGCCCTGCAGCTAATTAATTAAGCTACAAATAGTTT
  61  CGTTTTCACCTTGTCTAATAACTAATTAATTAAGGATCCCCCGTACCGGGCCCCCCCTCG
 121  AGGTCGACTTCTTTATTCTATACTTAAAAAGTGAAAATAAATACAAAGGTTCTTGAGGGT
 181  TGTGTTAAATTGAAAGCGAGAAATAATCATAAATTATTTCATTATCGCGATATCCGTTAA
 241  GTTTGTATCGTAATGGCAGAAGGATTTGCAGCCAATAGACAATGGATAGGACCAGAAGAA
 301  GCTGAAGAGTTATTAGATTTTGATATAGCAACACAAATGAGTGAAGAAGGACCACTAAAT
 361  CCAGGAGTAAACCCATTTAGGGTACCTGGAATAACAGAAAAAGAAAAGCAAAACTACTGT
 421  AACATATTACAACCTAAGTTACAAGATCTAAGGAACGAAATTCAAGAGGTAAAACTGGAA
 481  GAAGGAAATGCAGGTAAGTTTAGAAGAGCAAGATTTTTAAGGTATTCTGATGAACAAGTA
 541  TTGTCCCTGGTTCATGCGTTCATAGGATATTGTATATATTTAGGTAATCGAAATAAGTTA
 601  GGATCTTTAAGACATGACATTGATATAGAAGCACCCCAAGAAGAGTGTTATAATAATAGA
 661  GAGAAGGGTACAACTGACAATATAAAATATGGTAGACGATGTTGCCTAGGAACGGTGACT
 721  TTGTACCTGATTTTATTTATAGGATTAATAATATATTCACAGACAACCAACGCTCAGGTA
 781  GTATGGAGACTTCCACCATTAGTAGTCCCAGTAGAAGAATCAGAAATAATTTTTTGGGAC
 841  TGTTGGGCACCAGAAGAACCCGCCTGTCAGGACTTTCTTGGGGCAATGATACATCTAAAA
 901  GCTAAGACAAATATAAGTATACGAGAGGGACCTACCTTGGGGAATTGGACTAGAGAAATA
 961  TGGGCAACATTATTCAAAAAGGCTACTAGACAATGTAGAAGAGGCAGAATATGGAAAAGA
1021  TGGAATGAGACTATAACAGGACCATCAGGATGTGCTAATAACACATGTTATAATGTTTCA
1081  GTAATAGTACCTGATTATCAGTGTTATTTAGATAGAGTAGATACTTGGTTACAAGGGAAA
1141  ATAAATATATCATTATGTCTAACAGGAGGAAAAATGTTGTACAATAAAGTTACAAAACAA
1201  TTAAGCTATTGTACAGACCCATTACAAATCCCACTGATCAATTATACATTTGGACCTAAT
1261  CAAACATGTATGTGGAATACTTCACAAATTCAGGACCCTGAAATACCAAAATGTGGATGG
1321  TGGAATCAAATGGCCTATTATAACAGTTGTAAATGGGAAGAGGCAAAGGTAAAGTTTCAT
1381  TGTCAAAGAACACAGAGTCAGCCTGGATCATGGCGTAGAGCAATCTCGTCATGGAAACAA
1441  AGAAATAGATGGGAGTGGAGACCAGATTTGGAAAGTAAAAAGGTGAAAATATCTCTACAG
1501  TGCAATAGCACAAAAAACCTAACCTTTGCAATGAGAAGTTCAGGAGATTATGGAGAAGTA
1561  ACGGGAGCTTGGATAGAGTTTGGATGTCATAGAAATAAATCAAAACATCATTCTGAAGCA
1621  AGGTTTAGAATTAGATGTAGATGGAATGTAGGATCCGATACCTCGCTCATTGATACATGT
1681  GGAAACACTCGAGATGTTTCAGGTGCGAATCCTGTAGATTGTACCATGTATTCAAATAAA
1741  ATGTACAATTGTTCTTTACAAAATGGGTTTACTATGAAGGTAGATGACCTTATTGTGCAT
1801  TTCAATATGACAAAAGCTGTAGAAATGTATAATATTGCTGGAATTGGTCTTGTACATCT
1861  GACTTGCCATCGTCATGGGGGTATATGAATTGTAATTGTACAAATAGTAGTAGTAGTTAT
1921  AGTGGTACTAAAATGGCATGTCCTAGCAATCGAGGCATCTTAAGGAATTGGTATAACCCA
1981  GTAGCAGGATTACGACAATCCTTAGAACAGTATCAAGTTGTAAAACAACCAGATTACTTA
2041  GTGGTCCCAGAGGAAGTCATGGAATATAAACCTAGAAGGAAAAGGGCAGCTATTCATGTT
2101  ATGTTGGCTCTTGCAACAGTATTATCTATTGTCGGTGCAGGGACGGGGGCTACTGCTATA
2161  GGGATGGTAACCCAATACCACCAAGTTCTGGCAACCCATCAAGAAGCTATAGAAAAGGTG
2221  ACTGAAGCCTTAAAGATAAACAACTTAAGATTAGTTACATTAGAGCATCAAGTACTAGTA
2281  ATAGGATTAAAAGTAGAAGCTATGGAAAAATTCTTATATACAGCTTTCGCTATGCAAGAA
2341  TTAGGATGTAATCAAAATCAATTCTTCTGCAAAATCCCTCCTGGGTTGTGGACAAGGTAT
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FIG. 7 (cont'd)

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2401 AATATGACTATAAATCAAACAATATGGAATCATGGAAATATAACTTTGGGGGAATGGTAT
2461 AACCAAACAAAAGATTTACAACAAAAGTTTTATGAAATAATAATGGACATAGAACAAAAT
2521 AATGTACAAGGGAAAACAGGGATACAACAATTACAAAAGTGGGAAGATTGGGTAGGATGG
2581 ATGGGAAATATTCCACAATATTTAAAGGGACTATTGGGAGGTATCTTGGGAATAGGATTA
2641 GGAGTGTTATTATTGATTTTATGTTTACCTACATTGGTTGATTGTATAAGAAATTGTATC
2701 CACAAGATACTAGGATACACAGTAATTGCAATGCCTGAAGTAGAAGGAGAAGAAATACAA
2761 CCACAAATGGAATTGAGGAGAAATGGTAGGCAATGTGGCATGTCTGAAAAAGAGGAGGAA
2821 TGAATCGATACATCATGCAGTGGTTAAACAAAAACATTTTTATTCTCAAATGAGATAAAG
2881 TGAAAATATATATCATTATATTACAAAGTACAATTATTTAGGTTTAATCATGGGGAATGG
2941 ACAGGGGCGAGATTGGAAAATGGCCATTAAGAGATGTAGTAATGTTGCTGTAGGAGTAGG
3001 GGGGAAGAGTAAAAAATTTGGAGAAGGGAATTTCAGATGGGCCATTAGAATGGCTAATGT
3061 ATCTACAGGACGAGAACCTGGTGATATACCAGAGACTTTAGATCAACTAAGGTTGGTTAT
3121 TTGCGATTTACAAGAAGAAGAGAAAAATTTGGATCTAGCAAAGAAATTGATATGGCAAT
3181 TGTGACATTAAAAGTCTTTGCGGTAGCAGGACTTTTGAATATGACGGTGTCTACTGCTGC
3241 TGCAGCTGAAAATATGTATTCTCAAATGGGATTAGACACTAGGCCATCTATGAAAGAAGC
3301 AGGTGGAAAAGAGGAAGGCCCTCCACAGGCATATCCTATTCAAACAGTAAATGGAGTACC
3361 ACAATATGTAGCACTTGACCCAAAAATGGTGTCCATTTTCATGGAAAAGGCAAGAGAAGG
3421 ACTAGGAGGGGAGGAAGTTCAACTATGGTTTACTGCCTTCTCTGCAAATTTAACACCTAC
3481 TGACATGGCCACATTAATAATGGCCGCACCAGGGTGCGCTGCAGATAAAGAAATATTGGA
3541 TGAAAGCTTAAAGCAACTGACAGCAGAATATGATCGCACACATCCCCCTGATGCTCCCAG
3601 ACCATTACCCTATTTTACTGCAGCAGAAATTATGGGTATAGGATTAACTCAAGAACAACA
3661 AGCAGAAGCAAGATTTGCACCAGCTAGGATGCAGTGTAGAGCATGGTATCTCGAGGCATT
3721 AGGAAAATTGGCTGCCATAAAAGCTAAGTCTCCTCGAGCTGTGCAGTTAAGACAAGGAGC
3781 TAAGGAAGATTATTCATCCTTTATAGACAGATTGTTTGCCCAAATAGATCAAGAACAAAA
3841 TACAGCTGAAGTTAAGTTATATTTAAAACAGTCATTAAGCATAGCTAATGCTAATGCAGA
3901 CTGTAAAAAGGCAATGAGCCACCTTAAGCCAGAAAGTACCCTAGAAGAAAAGTTGAGAGC
3961 TTGTCAAGAAATAGGCTCACCAGGATATAAAATGCAACTCTTGGCAGAAGCTCTTACAAA
4021 AGTTCAAGTAGTGCAATCAAAAGGATCAGGACCAGTGTGTTTAATTGTAAAAAACCAGG
4081 ACATCTAGCAAGACAATGTAGAGAAGTGAAAAAATGTAATAAATGTGGAAAACCTGGTCA
4141 TCTAGCTGCCAAATGTTGGCAAGGAAATAGAAAGAATTCGGGAAACTGGAAGGCGGGGCG
4201 AGCTGCAGCCCCAGTGAATCAAATGCAGCAAGCAGTAATGCCATCTGCACCTCCAATGGA
4261 GGAGAAACTATTGGATTTATAAATTATAATAAAGTAGGTACGACTACAACATTAGAAAAG
4321 AGGCCAGAAATACTTATATTTGTAAATGGATATCCTATAAAATTTTTATTAGATACAGGA
4381 GCAGATATAACAATTTTAAATAGGAGAGATTTTCAAGTAAAAAATTCTATAGAAAATGGA
4441 AGGCAAAATATGATTGGAGTAGGAGGAGGAAAGAGAGGAACAAATTATATTAATGTACAT
4501 TTAGAGATTAGAGATGAAAATTATAAGACACAATGTATATTTGGTAATGTTTGTGTCTTA
4561 GAAGATAACTCATTAATACAACCATTATTGGGGAGAGATAATATGATTAAATTCAATATT
4621 AGGTTAGTAATGGCTCAATAATTTTATCCCGGGTTTTTATAGCTAATTAGTCATTTTTCG
4681 TAAGTAAGTATTTTTATTTAATACTTTTTATTGTACTTATGTTAAAT
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FIG. 8   Predicted nucleotide sequence of vCP329 insertion. The
          H6 promoter starts at position 2146. The coding sequence
          for FIV 97TM is from position 2022 to position 42. The
          I3L promoter starts at position 2253. The FIV <u>gag</u> start
          codon is at position 2353 and the <u>pol</u> stop codon is at
          position 3766.

```
   1    TTAATCAATAAAAAGAATTCCTGCAGGAATTCATAAAAATCATTCTTCTCCTTCTACTTC
  61    AGGCATTGCAATTACTGTGTATCCTAGTATCTTGTGGATACAATTTCTTATACAATCAAC
 121    CAATGTAGGTAAACATAAAATCAATAATAACACTCCTAATCCTATTCCCAAGATACCTCC
 181    CAATAGTCCCCTTTTCCTTCTAGGTTTATATTCCATGACTTCCTCTGGGACCACTAAGTA
 241    ATCTGGTTGTTTTACAACTTGATACTGTTCTAAGGATTGTCGTAATCCTGCTACTGGGTT
 301    ATACCAATTCCTTAAGATGCCTCGATTGCTAGGACATGCCATTTTAGTACCACTATAACT
 361    ACTACTACTATTTGTACAATTACAATTCATATACCCCCATGACGATGGCAAGTCAGATGT
 421    ACAAGACCAATTTCCAGCAATATTATACATTTCTACAGCTTTTGTCATATTGAAATGCAC
 481    AATAAGGTCATCTACCTTCATAGTAAACCCATTTTGTAAAGAACAATTGTACATTTTATT
 541    TGAATACATGGTACAATCTACAGGATTCGCACCTGAAACATCTCGAGTGTTTCCACATGT
 601    ATCAATGAGCGAGGTATCGGATCCTACATTCCATCTACATCTAATTCTAAACCTTGCTTC
 661    AGAATGATGTTTTGATTTATTTCTATGACATCCAAACTCTATCCAAGCTCCCGTTACTTC
 721    TCCATAATCTCCTGAACTTCTCATTGCAAAGGTTAGGTTTTTTGTGCTATTGCACTGTAG
 781    AGATATTTTCACCTTTTTACTTTCCAAATCTGGTCTCCACTCCCATCTATTTCTTTGTTT
 841    CCATGACGAGATTGCTCTACGCCATGATCCAGGCTGACTCTGTGTTCTTTGACAATGAAA
 901    CTTTACCTTTGCCTCTTCCCATTTACAACTGTTATAATAGGCCATTTGATTCCACCATCC
 961    ACATTTTGGTATTTCAGGGTCCTGAATTTGTGAAGTATTCCACATACATGTTTGATTAGG
1021    TCCAAATGTATAATTGATCAGTGGGATTTGTAATGGGTCTGTACAATAGCTTAATTGTTT
1081    TGTAACTTTATTGTACAACATTTTTCCTCCTGTTAGACATAATGATATATTTATTTTCCC
1141    TTGTAACCAAGTATCTACTCTATCTAAATAACACTGATAATCAGGTACTATTACTGAAAC
1201    ATTATAACATGTGTTATTAGCACATCCTGATGGTCCTGTTATAGTCTCATTCCATCTTTT
1261    CCATATTCTGCCTCTTCTACATTGTCTAGTAGCCTTTTTGAATAATGTTGCCCATATTTC
1321    TCTAGTCCAATTCCCCAAGGTAGGTCCCTCTCGTATACTTATATTTGTCTTAGCTTTTAG
1381    ATGTATCATTGCCCCAAGAAAGTCCTGACAGGCGGGTTCTTCTGGTGCCCAACAGTCCCA
1441    AAAAATTATTTCTGATTCTTCTACTGGGACTACTAATGGTGGAAGTCTCCATACTACCTG
1501    AGCGTTGGTTGTCTGTGAATATATTATTAATCCTATAAATAAAATCAGGTACAAAGTCAC
1561    CGTTCCTAGGCAACATCGTCTACCATATTTTATATTGTCAGTTGTACCCTTCTCTCTATT
1621    ATTATAACACTCTTCTTGGGGTGCTTCTATATCAATGTCATGTCTTAAAGATCCTAACTT
1681    ATTTCGATTACCTAAATATATACAATATCCTATGAACGCATGAACCAGGGACAATACTTG
1741    TTCATCAGAATACCTTAAAAATCTTGCTCTTCTAAACTTACCTGCATTTCCTTCTTCCAG
1801    TTTTACCTCTTGAATTTCGTTCCTTAGATCTTGTAACTTAGGTTGTAATATGTTACAGTA
1861    GTTTTGCTTTTCTTTTTCTGTTATTCCAGGTACCCTAAATGGGTTTACTCCTGGATTTAG
1921    TGGTCCTTCTTCACTCATTTGTGTTGCTATATCAAAATCTAATAACTCTTCAGCTTCTTC
1981    TGGTCCTATCCATTGTCTATTGGCTGCAAATCCTTCTGCCATTACGATACAAACTTAACG
2041    GATATCGCGATAATGAAATAATTTATGATTATTTCTCGCTTTCAATTTAACACAACCCTC
2101    AAGAACCTTTGTATTTATTTTCACTTTTTAAGTATAGAATAAAGAACTGCAGCTAATTAA
2161    TTAAGCTACAAATAGTTTCGTTTTCACCTTGTCTAATAACTAATTAATTAAGGATCCCCC
2221    GTACCGGGCCCCCCCTCGAGGTCGACATCGATACATCATGCAGTGGTTAAACAAAAACAT
2281    TTTTATTCTCAAATGAGATAAAGTGAAAATATATATCATTATATTACAAAGTACAATTAT
2341    TTAGGTTTAATCATGGGGAATGGACAGGGGCGAGATTGGAAAATGGCCATTAAGAGATGT
2401    AGTAATGTTGCTGTAGGAGTAGGGGGGAAGAGTAAAAAATTTGGAGAAGGGAATTTCAGA
2461    TGGGCCATTAGAATGGCTAATGTATCTACAGGACGAGAACCTGGTGATATACCAGAGACT
2521    TTAGATCAACTAAGGTTGGTTATTTGCGATTTACAAGAAAGAAGAGAAAAATTTGGATCT
2581    AGCAAAGAAATTGATATGGCAATTGTGACATTAAAAGTCTTTGCGGTAGCAGGACTTTTG
2641    AATATGACGGTGTCTACTGCTGCTGCAGCTGAAAATATGTATTCTCAAATGGGATTAGAC
2701    ACTAGGCCATCTATGAAAGAAGCAGGTGGAAAAGAGGAAGGCCCTCCACAGGCATATCCT
2761    ATTCAAACAGTAAATGGAGTACCACAATATGTAGCACTTGACCCAAAAAATGGTGTCCATT
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FIG. 8 (cont'd)

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2821 TTCATGGAAAAGGCAAGAGAAGGACTAGGAGGGGAGGAAGTTCAACTATGGTTTACTGCC
2881 TTCTCTGCAAATTTAACACCTACTGACATGGCCACATTAATAATGGCCGCACCAGGGTGC
2941 GCTGCAGATAAAGAAATATTGGATGAAAGCTTAAAGCAACTGACAGCAGAATATGATCGC
3001 ACACATCCCCCTGATGCTCCCAGACCATTACCCTATTTTACTGCAGCAGAAATTATGGGT
3061 ATAGGATTAACTCAAGAACAACAAGCAGAAGCAAGATTTGCACCAGCTAGGATGCAGTGT
3121 AGAGCATGGTATCTCGAGGCATTAGGAAAATTGGCTGCCATAAAAGCTAAGTCTCCTCGA
3181 GCTGTGCAGTTAAGACAAGGAGCTAAGGAAGATTATTCATCCTTTATAGACAGATTGTTT
3241 GCCCAAATAGATCAAGAACAAAATACAGCTGAAGTTAAGTTATATTTAAAACAGTCATTA
3301 AGCATAGCTAATGCTAATGCAGACTGTAAAAAGGCAATGAGCCACCTTAAGCCAGAAAGT
3361 ACCCTAGAAGAAAGTTGAGAGCTTGTCAAGAAATAGGCTCACCAGGATATAAAATGCAA
3421 CTCTTGGCAGAAGCTCTTACAAAAGTTCAAGTAGTGCAATCAAAAGGATCAGGACCAGTG
3481 TGTTTTAATTGTAAAAAACCAGGACATCTAGCAAGACAATGTAGAGAAGTGAAAAAATGT
3541 AATAAATGTGGAAAACCTGGTCATCTAGCTGCCAAATGTTGGCAAGGAAATAGAAAGAAT
3601 TCGGGAAACTGGAAGGCGGGGCGAGCTGCAGCCCCAGTGAATCAAATGCAGCAAGCAGTA
3661 ATGCCATCTGCACCTCCAATGGAGGAGAAACTATTGGATTTATAAATTATAATAAAGTAG
3721 GTACGACTACAACATTAGAAAAGAGGCCAGAAATACTTATATTTGTAAATGGATATCCTA
3781 TAAAATTTTTATTAGATACAGGAGCAGATATAACAATTTTAAATAGGAGAGATTTTCAAG
3841 TAAAAAATTCTATAGAAAATGGAAGGCAAAATATGATTGGAGTAGGAGGAGGAAAGAGAG
3901 GAACAAATTATATTAATGTACATTTAGAGATTAGAGATGAAAATTATAAGACACAATGTA
3961 TATTTGGTAATGTTTGTGTCTTAGAAGATAACTCATTAATACAACCATTATTGGGGAGAG
4021 ATAATATGATTAAATTCAATATTAGGTTAGTAATGGCTCAATAATTTTATCCCGGGTTTT
4081 TATAGCTAATTAGTCATTTTCGTAAGTAAGTATTTTTATTTAATACTTTTTATTGTACT
4141 TATGTTAAAT
```

A.	CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :Please See Extra Sheet.
US CL   :424/69.1, 172.3, 235.1, 236, 320.1; 424/188.1, 199.1, 232.1, 93.2
According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/69.1, 172.3, 235.1, 236, 320.1; 424/188.1, 199.1, 232.1, 93.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

AIDSLINE, MEDLINE, WPIDS, USPATFUL

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	FRANCHINI et al. Highly attenuated HIV type 2 recombinant poxviruses, but not HIV-2 recombinant Salmonella vaccines, induce long-lasting protection in rhesus macaques. AIDS Res. Human Retro. 1995, Vol. 11, No. 8, pages 909-920, see entire document.	1-3, 5-9, 13, 19-30 ------------- 4, 10, 11, 14-18
X ----- Y	COX et al. Induction of cytotoxic T lymphocytes by recombinant canarypox (ALVAC) and attenuated vaccinia (NYVAC) viruses expressing the HIV-1 envelope glycoprotein. Virol. 1993, Vol. 195, pages 845-850, see entire document.	1-3, 5-9, 12, ------------ 4, 10, 11, 14-18

[X]	Further documents are listed in the continuation of Box C.	[ ]	See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 JANUARY 1998	0 6 MAR 1998

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WARDLEY et al. The use of feline herpesvirus and baculovirus as vaccine vectors for the gag and env genes of feline leukaemia virus. J. Gen. Virol. 1992, Vol. 73, pages 1811-1818, see entire document.	4
Y	PINCUS et al. Poxvirus-based vectors as vaccine candidates. Biologicals. 1995, Vol. 23, pages 159-164, see entire document.	10
Y	OKUDA et al. Induction of potent humoral and cell-mediated immune responses following direct injection of DNA encoding the HIV type 1 env and rev gene products. AIDS Res. Human Retro. 1995, Vol. 11, No. 8, pages 933-943, see entire document.	11
Y	GONDA et al. Bovine immunodeficiency virus: molecular biology and virus-host interactions. Virus Res. 1994, Vol. 32, pages 155-181, see entire document.	14
Y	OLMSTED et al. Molecular cloning of feline immunodeficiency virus. Proc. Natl. Acad. Sci. USA. April 1989, Vol. 86, pages 2448-2452, see entire document.	15
Y	WHETTER et al. Equine infectious anemia virus derived from a molecular clone persistently infects horses. J. Virol. December 1990, Vol. 64, No. 12, pages 5750-5756, see entire document.	16
Y	ANDRESSON et al. Nucleotide sequence and biological properties of a pathogenic proviral molecular clone of neurovirulent visna virus. Virol. 1993, Vol. 193, pages 89-105, see entire document.	17
Y	SALTARELLI et al. Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. Virol. 1990, Vol. 179, pages 347-364, see entire document.	18

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12P 21/06; C12N 15/00, 7/00, 7/04; A61K 39/21, 39/12, 39/275; A01N 63/00